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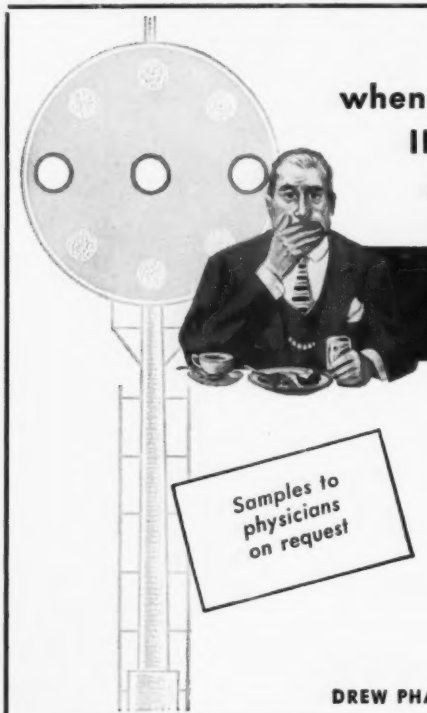

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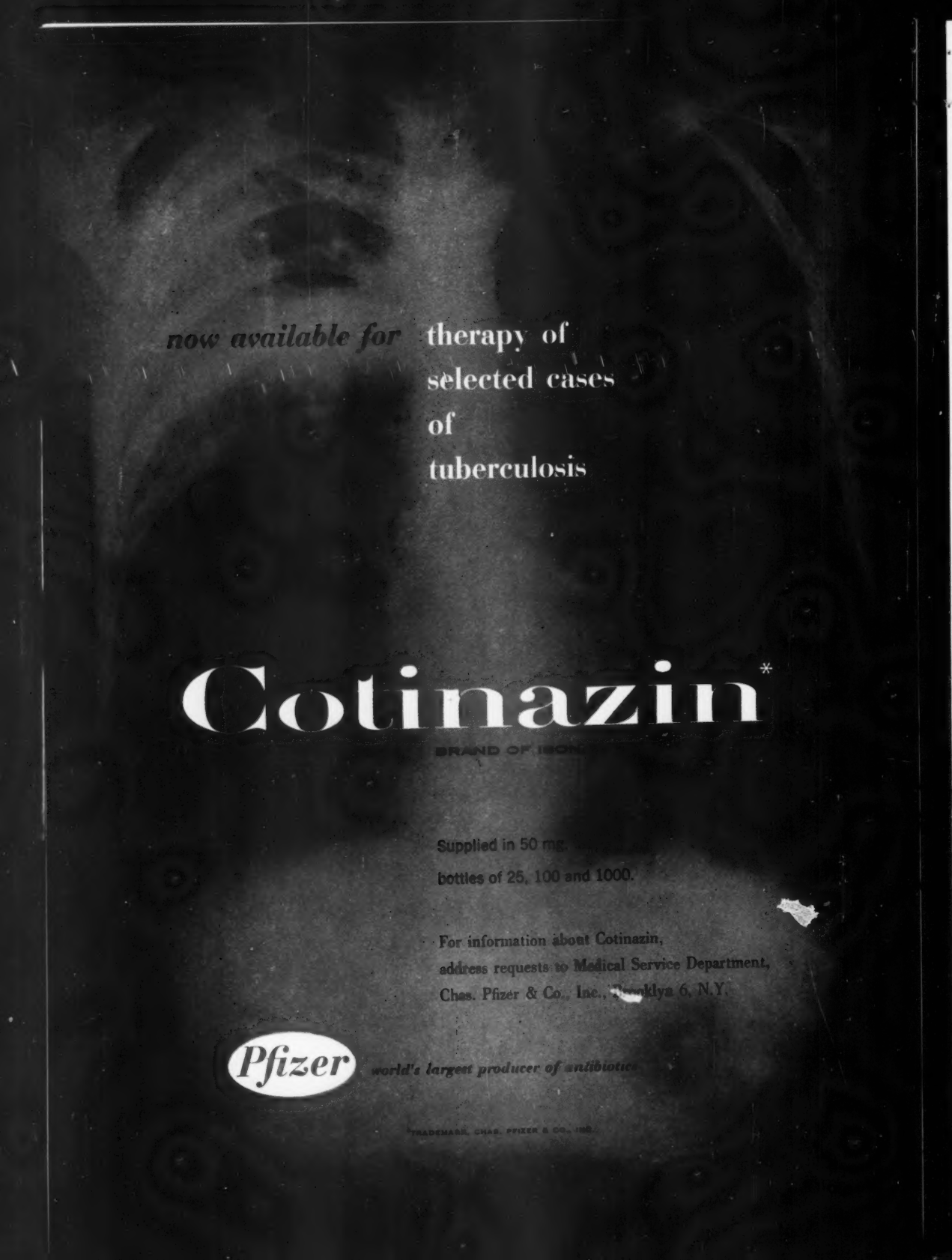
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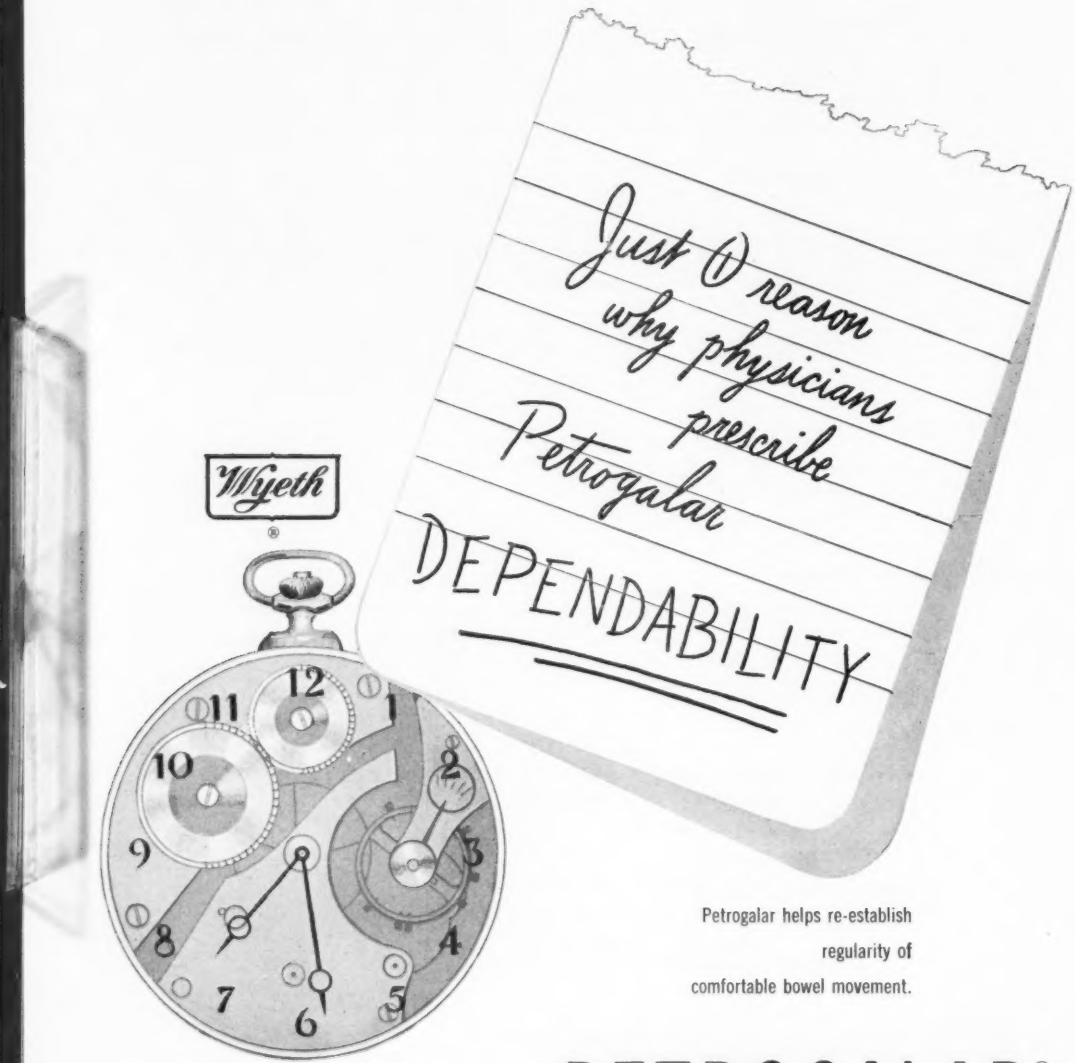

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A SIMPLE DIETARY TECHNIQUE FOR THE PRODUCTION OF HEPATIC NECROSIS WITH A CONSIDERATION OF THE FACTORS PREVENTING THE LESION

JOSEPH GILLMAN, CHRISTINE GILBERT, THEODORE GILLMAN AND ISOBEL SPENCE, Johannesburg, S. Africa.

THE OBJECT of this communication is to describe a simple dietary technique for the consistent production of necrosis of the liver in rats. In view of the limited number of nutrients in our experimental diet, we have been able to examine some of the conditions required for enhancing or reducing the incidence of hepatic necrosis.

We shall draw attention to the paradoxical situation arising from this study where two simple diets, equally lacking in vitamin E and in methionine, lead to a high and a low incidence of necrosis of the liver respectively. Nutritional investigators frequently assert that lack of methionine or of vitamin E is the cause of hepatic necrosis in rats fed a low protein diet. However, we shall confirm the point of view originally presented, namely, that the importance of a particular food factor in preventing a pathological process is dependent not only upon the intrinsic nutritional "needs" of the organism but also on the type of metabolism fostered in the organism by the constituents of the diet (Gillman and Gillman, 1951).

MATERIAL AND METHODS

The necrosis-producing diet consisted of 10 per cent food yeast and 90 per cent dextrinised potato starch. The yeast used in our experiments was of two varieties, namely a food yeast and a killed dried brewer's yeast, both of which are manufactured in South Africa. The chemical analyses of the food yeast (Dehydrated Products Ltd.) and of the brewer's yeast (South African Food Extracts Ltd.) are appended herewith.

	*Food Yeast** (Dehydrated Products Ltd.)	*Brewer's Yeast* (S. A. Food Extracts Ltd.)
Solid content	94-95%	100%
Total nitrogen	7.2%	7.83%
Fat	2.3%	1.78%
Thiamine	20- 30 gamma per gram	32 gamma per gram
Riboflavin	50- 60 gamma per gram	58 gamma per gram
Nicotinic acid	400-500 gamma per gram	680 gamma per gram
Methionine	0.65%	0.79%
Cystine	0.88%	0.45%
Alpha-tocopherol	NIL	NIL
Iron	18-20 mg. per 100 grams	Traces
Total ash		8.8%

The potato starch was of American manufacture and was obtained from Manischewitz Food Products Incorporated. The average analysis of their Idaho potato starch was as follows:

Moisture (max.)	18.00%
Ash	0.20%
Fibre	0.02%
Nitrogen free extract	81.78%

*Food yeast was supplied to us by Dehydrated Products Ltd. and by the Industrial Development Corporation of South Africa Ltd. The thiamine and riboflavin contents of both yeasts were closely similar but the nicotinic acid content of the Industrial Development Corporation yeast was lower than that of the Dehydrated Products Food yeast.

Departments of Physiology and Anatomy and Joint Nutrition Research Unit of the Council for Scientific and Industrial Research and of the University of the Witwatersrand.

A standard procedure was adopted in preparing the food yeast and potato starch diet. In our early experiments, 90 gm. of the potato starch were added to boiling water and stirred continuously until a thick, clear, jelly-like product was formed. After allowing the starch to cool, 10 gm. of food yeast (or brewer's yeast) were thoroughly mixed into the starch. Portions of the mixture were then fed to the rats.

When prepared in the manner just described, we found the mixture difficult to handle; in our later experiments, therefore, the technique of preparing the diet was slightly modified. A variable quantity of potato starch was boiled with water and, after being converted into a gel, was allowed to cool. Thereafter, the starch was dried slowly in a low temperature oven and when thoroughly dry, it was ground into a fine powder. To 90 gm. of the dry powder, 10 gm. of food yeast (or brewer's yeast) were then added. The dry mixture was moistened with water before feeding to the rats.

Except where specifically stated all the rats weighed between 30 and 40 gm. at the commencement of the experiment. A minimum of ten rats was used for each experiment, and, as far as possible, the sexes were equally represented. A total of 324 rats was used in the present investigation.

For statistical analysis of the data,* the 95% limits of the incidence of necrosis (p) in each group were determined from the tables of Hald (1948). In comparing two groups, a statistically significant difference was assessed on the basis of non-overlap of the two ranges of necrosis incidence, and this significance has a 95% probability of assurance attached to it.

RESULTS

Efficiency of the diet in producing necrosis of the liver.

The nature of the liver lesion in our rats is indistinguishable from that which Himsworth and Glynn (1944), Glynn and Himsworth (1944) and Himsworth (1945) have described as generalised massive necrosis and as partial massive necrosis (see Figs. 1-6). In our rats, the acute stages of the necrosis are manifest microscopically as anoxic necrosis in some livers (Figs. 7 & 8) and as anoxic necrosis, associated with engorgement of the sinusoids, simulating haemorrhagic necrosis, in others (Fig. 9). In the later stages of the experiment, the pathogenesis of post-necrotic scarring is evident (Figs. 10, 11 & 12) and even complete lobar absorption is not uncommon. Indeed, in one and the same liver, it is possible to find all stages in the pathogenesis of partial massive necrosis. Thus, in Fig. 4, three stages in haemorrhagic partial necrosis and even the early stages of post-necrotic scarring are evident. In Fig. 3 post-necrotic scarring and nodular hyperplasia can be seen in the right and left median

*We are indebted to Dr. S. Brenner for his statistical analysis of the data.

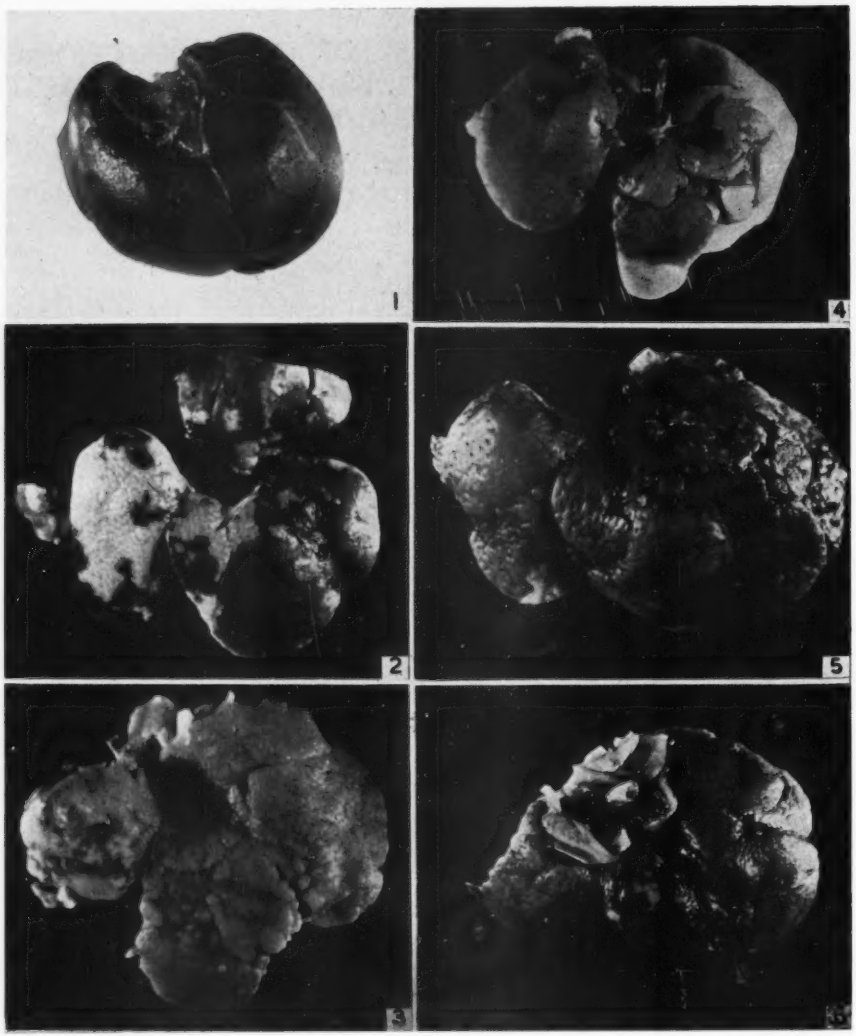


Fig. 1: Normal liver of control rat fed a stock diet.

Fig. 2: Liver of a rat fed potato starch and food yeast showing the distribution of anoxic and haemorrhagic necrosis.

Fig. 3: Extensive post-necrotic scarring with a sub-acute lesion on the right lateral lobe.

Fig. 4: Several stages in necrosis from the haemorrhagic form to early post-necrotic scarring evident in different lobes of the same liver.

Fig. 5: Early post-necrotic scarring in the left and right median lobes and various stages of necrosis in the right lateral lobes of the liver.

Fig. 6: Liver showing predominant localisation of the lesion in the right median and right lateral lobes with scarring on the left median.

lobes and in the left lateral lobe, with atrophy and diffuse hypertrophy in the right upper lateral and general hypertrophy and nodular hyperplasia in the left lateral lobe.

From this it follows that the liver may undergo massive necrosis during a single episode, or it may be progressively destroyed by a series of episodes recurring at irregular intervals. In the latter instance, it is more

than likely that a diversity of lesions will be found in a single liver. In our rats, acute necrosis was observed with equal frequency in the right median, right lower lateral and left median lobes. Gross atrophy was predominantly a feature of the left median lobe.

Himsworth and Glynn, however, draw attention to the localization of the lesion in the left lobe of the liver in their experimental rats and suggest that this is dependent either on a noxious substance conveyed to the left lobe of the liver by the blood from the spleen and large intestine or that blood from the small intestine contains a substance which prevents the liver cells in the right lobes from developing necrosis. As we have seen, the lesions in our rats are not confined to any particular lobe of the liver. If the gut and "streamlining" of portal blood flow do play a part in modulating the distribution of liver necrosis, then this role is not apparent in our rats.

Time of death and incidence of necrosis in relation to initial weight of the rat.

With the exception of a small number of rats which were killed on specified days during the course of the experiment, all the rats described in this study were allowed to die or were killed only when moribund. The percentage of rats of different weights dying at various times during the experiment is presented in Fig. 13A.

It is evident that all rats on a diet of potato starch and food yeast, weighing 30-40 gm. at the outset of the experiment, are dead by the 105th day. Of this group, 64% are dead by the 50th day and 90% by the 65th day. At autopsy necrosis of the liver was found in 93 of 97 rats of this weight group giving a 'p' value of 0.959 with 95 per cent limits of 0.904-0.989 (Fig. 13B and Table 2).

In another group of rats fed on the potato starch and food yeast diet and killed at specified intervals, liver necrosis occurred in some rats as early as the 23rd day and was delayed in others for as long as 45 or 50 days. It should be pointed out, however, that although necrosis may develop at an early stage in the experiment and may involve a considerable part of a lobe of the liver, the animal may not succumb. At a later stage, another lobe of the liver may be destroyed and still without fatal consequences. Indeed, the liver may be subjected to a succession of gross injuries before death supervenes.

On the other hand, when several lobes of the liver undergo necrosis simultaneously, the animal may die suddenly. Such acute massive necrosis may be found in rats dying at any stage during the experiment. In view of these findings, therefore, the wide diversity in the macroscopic and microscopic appearances of the livers as described and portrayed above is not entirely unexpected.

Reverting to Fig. 13A, it is evident that, whatever the weight of the rat at the commencement of the experiment, the potato starch and food yeast diet cannot sustain life indefinitely. The initial weight of the animal, however, does influence the duration of survival. Thus, rats with an initial weight of 200-250 gm. are all dead by the 245th day of the experiment and survived more than twice as long as those rats included in the 30-40 gm. weight group. Moreover, the earliest death in the 200-250 gm. group was recorded on the 105th day whereas the earliest death occurred on the 25th day in the 30-40 gm. group.

The incidence of necrosis in rats in the heavier weight groups (that is those rats having an initial weight of between 40 gm. and 250 gm.) was between 80% and 100% (Fig. 13B). In rats weighing over 300 gm., 33 per cent (3 out of 9) were dead by the 165th day; none of these rats showed liver necrosis. Of the 6 remaining rats sacrificed on the 197th day, liver necrosis was found in one.

From the above we can conclude that a diet of potato starch and food yeast is highly efficient in producing necrosis of the liver in rats having an initial weight of 250 gm. or less. The time of death as well as the onset of necrosis of the liver is a function of the weight of the animal and therefore of its age at the commencement of the experiment. Since the initial weight of the rat determines the time of onset of hepatic necrosis, it is clear that if 200 gm. rats are used, it is unlikely that necrosis of the liver will develop before the 100th day, nor is it likely that 50 per cent of the animals will develop necrosis before the 170th day (Fig. 13B).

Rats weighing between 30 and 40 gm. are therefore the most useful for the study of the factors promoting and preventing necrosis of the liver on the food yeast and potato starch diet: they develop necrosis not later than the 105th day of the experiment.

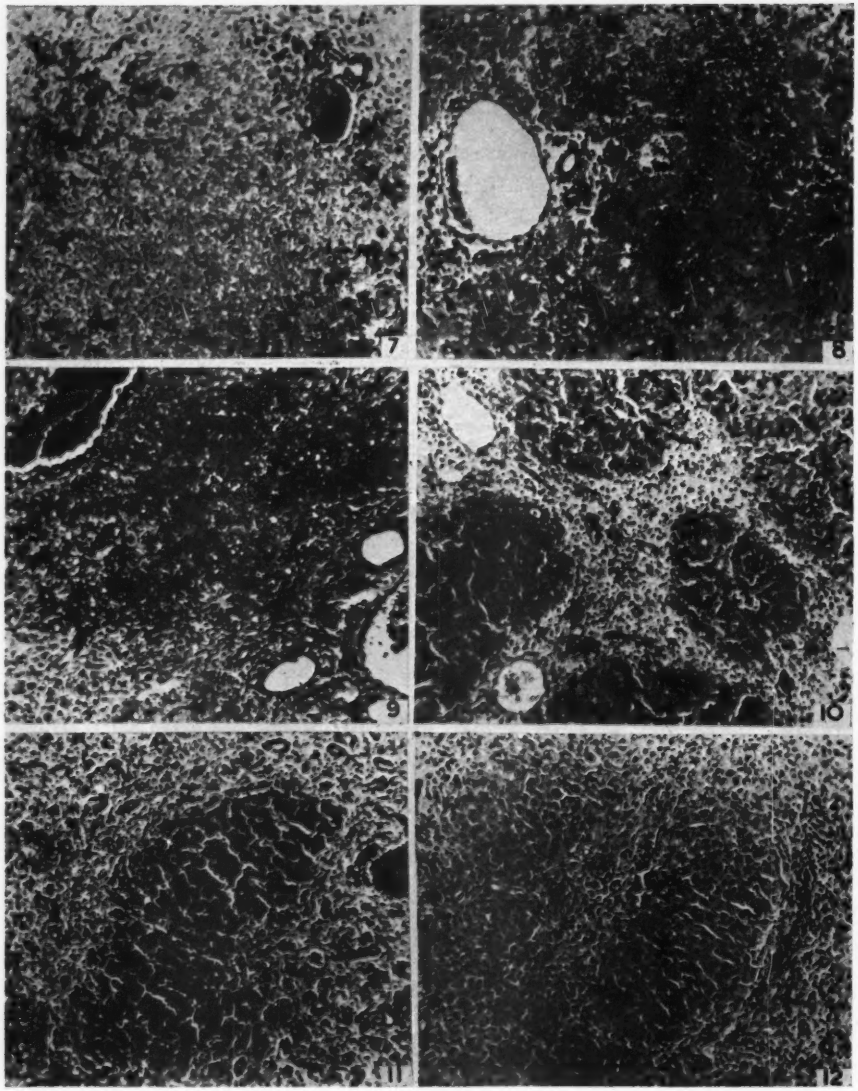
From a consideration of Fig. 13A we may also conclude that in every age group rats show considerable individual variation in the duration of survival. The longer the survival period the greater is the possibility that necrosis will develop. The shorter the survival period, within the limits specified, the more likely will there be a low incidence of hepatic necrosis. In this connection it should be added that we could not relate this individual difference in reactivity to any sex differences as seems to be the case in reactions in the liver elicited in other nutritional experiments (Nathanson and Zamecnik, 1947, Gyorgy *et al.*, 1947, 1949, and Emerson *et al.*, 1951).

TABLE 1

COMPARISON OF POTATO STARCH AND GLUCOSE AS A SOURCE OF CARBOHYDRATE IN THE PRODUCTION OF NECROSIS OF THE LIVER.

Diet	Number of animals n	Number of animals with necrosis of the liver m	Incidence of necrosis m/n = p	95% limits of p
Potato starch + 10% food yeast	97	93	0.959	0.904 to 0.989
Glucose + 10% food yeast	23	16	0.696	0.471 to 0.868

The incidence of hepatic necrosis is significantly lower in the glucose diet than in the potato starch diet.



Figs. 7 to 12 represent different stages in the pathogenesis of hepatic necrosis and post-necrotic scarring.

Fig. 7: Shows extensive anoxic necrosis involving an entire lobule.

Fig. 8. High power study of a portion of the liver shown in Fig. 7.

Fig. 9: Section of liver showing haemorrhagic necrosis extending from the fibrous portal tract to the sublobular vein.

Fig. 10: Early stage in hepatic fibrosis showing removal of liver debris and increase in fibroblastic elements with gross distortion of the liver architecture.

Fig. 11: Later stage than Fig. 10 with low grade bile duct hyperplasia, great increase in fibroblastic cells with survival of a small portion of a liver lobule.

Fig. 12: Gross distortion of liver architecture and great increase in fibrous tissue leading to post-necrotic scarring.

All rats on a diet of potato starch and food yeast lose weight steadily from the time of commencement of the experiment. The average percentage loss of weight in all animals, initially over 100 gm. and surviving until the 70th day, varies between 13 and 17 per cent. At the time of death the average loss of weight as per cent of the initial weight is 26 per cent in the 100-120 gm. group, 37 per cent for rats in the 200-250 gm. group and 29 per cent for those in the 300-350 gm. group.

We realize that, as a diet, potato starch and food yeast is devoid of many food factors essential for growth and survival. It does not necessarily follow, however, that a diet which is unable to promote growth will automatically evoke necrosis of the liver. These two phenomena can quite easily be disengaged.

and the time of survival.

Dietary factors modifying the incidence of necrosis

(a) *Nature of the carbohydrate.* If glucose is substituted for potato starch, all rats in the 30-40 gm. weight group are dead by the 85th day (Fig. 15A). However, the incidence of hepatic necrosis falls to 70 per cent as compared with 96 per cent in rats on the potato starch and food yeast diet (Fig. 15B). From Table 1 it can be seen that the 95 per cent limits of 'p' for the food yeast-glucose combination are between 0.471 and 0.868. Thus the incidence of necrosis in this group is significantly less than that calculated for the potato starch-food yeast diet. This difference in the necrosis-producing effect of glucose and potato starch when used in combination with food yeast cannot be explained.

When fed *uncooked* potato starch and food yeast, rats survive for a period of 21-32 days. None of the rats (total 10) developed necrosis of the liver and all showed great distention of the stomach and of the caecum at post-mortem.

Since the yeast is killed, it is unlikely that the bowel distention is a consequence of the fermentation of the potato starch by the yeast. The fact that the animals survived for at least 21 days indicates that some of the nutriment (either the potato starch or the food yeast or both) was absorbed. Since necrosis of the

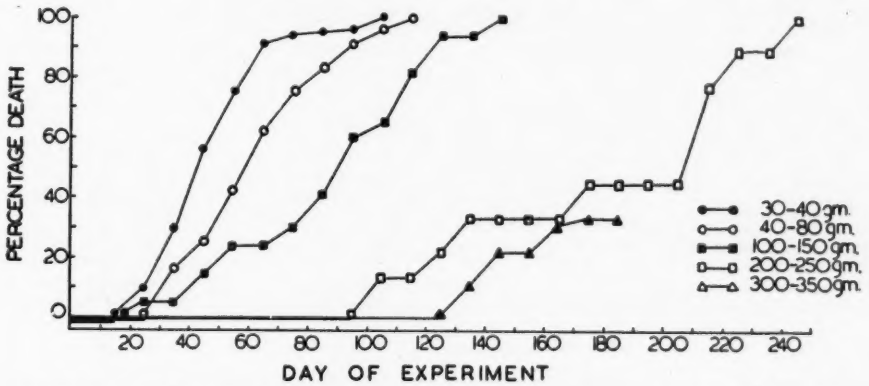


Fig. 13, A: Shows the cumulative incidence of death in relation to the duration of each experiment in rats of different initial weights and fed a diet of 90% potato starch and 10% food yeast. In the 300-350 gm. weight group the surviving rats were sacrificed on the 197th day of the experiment.

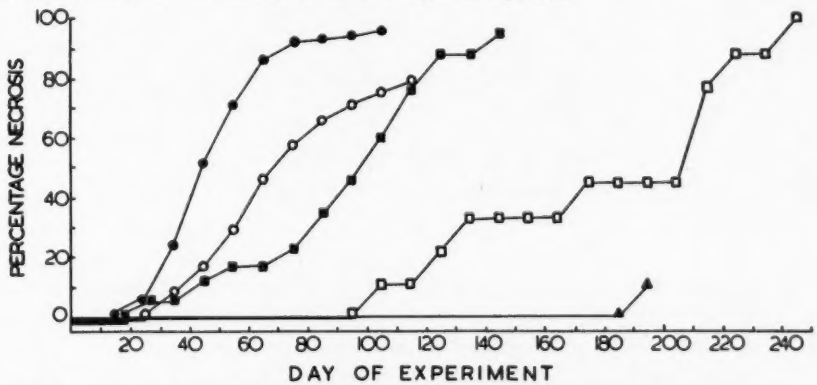


Fig. 13, B: Shows the cumulative incidence of hepatic necrosis as percentages of the initial total number of animals in each weight group in relation to the duration of each experiment.

liver is excluded when rats are fed uncooked starch with yeast, it is evident that the nature of the carbohydrate as well as the form in which it is presented to the animal play an important role in determining the incidence of hepatic necrosis.

(b) *Absence of food yeast.* If fed glucose alone or glucose to which is added 0.2 mg. thiamine or 0.2 mg. riboflavin per 100 gm. of food, in order to prolong survival, rats die between the 38th and 45th day of the experiment. At autopsy, none of 20 rats showed necrosis of the liver. It will be recalled, however, that on a diet of glucose and food yeast, 54 per cent of rats are dead by the 45th day of the experiment and of these, 74 per cent have developed necrosis of the liver, that is 40 per cent of the total group.

A diet of glucose alone or of glucose with thiamine or riboflavin is obviously lacking in those substances, such as vitamin E and methionine, usually regarded as protective against necrosis of the liver. Since necrosis of the liver does not develop, it follows that certain minimal nutrients must be *included* in the diet in order to evoke hepatic necrosis. These nutrients are obviously made available when 10 per cent food yeast is given in conjunction with dextrinised potato starch or with glucose. Therefore, necrosis is a function, in part at least, of the peculiar combination of factors within the food yeast.

(c) *Increasing the concentration of food yeast in the diet.* When potato starch is combined with 20 per cent instead of 10 per cent food yeast, the time of survival of the rats is slightly enhanced (Fig. 15A) while the incidence of necrosis of the liver is significantly reduced (Fig. 15B, Table 2).

(d) *Addition of Steenbock 40 salt mixture.* The addition of 3 per cent Steenbock 40 salt mixture to a diet of potato starch and 10 per cent food yeast shortens the duration of survival of the rats by half and 42% of animals develop necrosis of the liver (Fig. 15A, B). Statistically, therefore, the salt mixture has led to a significant reduction in the incidence of necrosis of the liver (Fig. 15B & Table 2). On a diet of potato starch and food yeast, 75 per cent of rats are dead by the 55th day, and the incidence of necrosis of the liver at this time is 70 per cent. If we assume that the surviving 25 per cent of rats had not as yet developed necrosis of the liver, the total incidence of necrosis of the liver would have been 70 per cent as compared with 42 per cent after the addition of salts. Clearly then, although a salt mixture may not be able entirely to protect the liver, it can be a factor modulating the incidence of necrosis and may determine whether or not a diet will be 100 per cent or 50 per cent efficient in promoting liver necrosis.

(e) *Addition of methionine.* The administration of 50 mg. methionine per rat per day enhances survival, the first rat dying on the 65th day of the experiment and the last on the 115th day (Fig. 14A). Moreover, methionine results in complete protection of the liver in all rats, giving the limits of 'p' between 0.000 and 0.336 (Fig. 14B & Table 2).

Full protection of the liver from the necrosis-producing effects of the diet was afforded when the methionine was added to the food as a dry crystalline powder. When the methionine was made up in watery suspension and the appropriate concentration of the fluid added daily to the food, all the animals developed necrosis of the liver. We are unable to account for this anomalous result.

(f) *Addition of choline.* The addition of 50 mg. of choline per rat per day not only shortens the time of survival of the rats but fails to protect the liver against necrosis (Fig. 14A & B & Table 2).

(g) *Addition of cystine.* When 50 mg. cystine are given to each rat per day, the animals survive for a period longer than observed in those rats receiving methionine daily (Fig. 14A & B). Although nine of

TABLE 2

THE EFFECT OF THE ADDITION OF VARIOUS DIETARY FACTORS TO A DIET OF POTATO STARCH + 10% FOOD YEAST ON THE INCIDENCE OF HEPATIC NECROSIS

Diet.	Addition to diet.	Number of animals. n	Period of experiment during which animals die. (Days)	Day of first appearance of necrosis.	Total no. of animals with hepatic necrosis. m	Incidence of hepatic necrosis. $m/n = p^*$	95% limits of p	
Potato starch & food yeast	—	97	25-105	25	93	0.959	0.904 to 0.989	
	10% food yeast	9	45-115	45	3	0.334	0.075 to 0.701	Significant protection of the liver.
	3% Steenbock 40 salt mixture	24	25-55	25	10	0.417	0.221 to 0.634	Significant protection of the liver.
	methionine 50 mg/rat/day	9	65-115	NIL	0	0.000	0.000 to 0.336	Significant protection of the liver.
	choline 50 mg/rat/day	10	25-65	15	10	1.000	0.692 to 1.000	No significant protection of the liver.
	cystine 50 mg/rat/day	10	45-135	75	9	0.900	0.555 to 0.997	No significant protection of the liver.
	alpha-tocopherol 50 mg/rat/day	45	35-145	NIL	0	0.000	0.000 to 0.079	Significant protection of the liver.
	dried thyroid 50 mg/rat/day	6	35-45	25	3	0.500	0.118 to 0.882	Significant protection of the liver.

*percentage incidence = 100 × p.

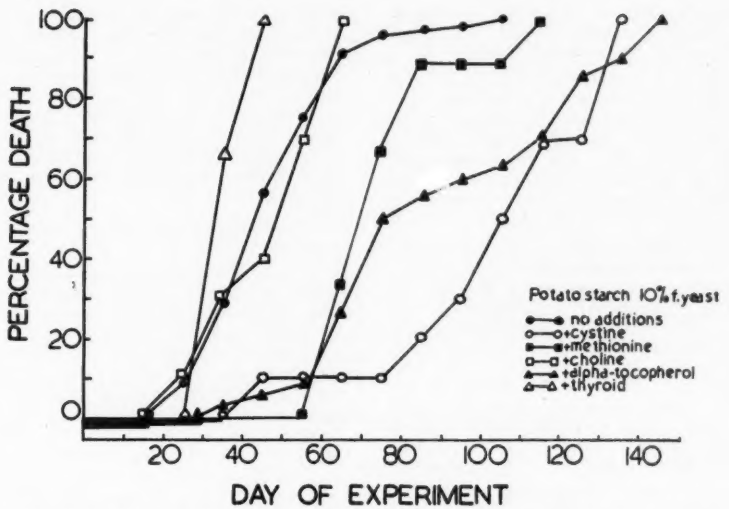


Fig. 14, A: Shows the cumulative incidence of death in relation to the duration of the experiment in rats fed potato starch (90%) and food yeast (10%) alone and with the supplements specified.

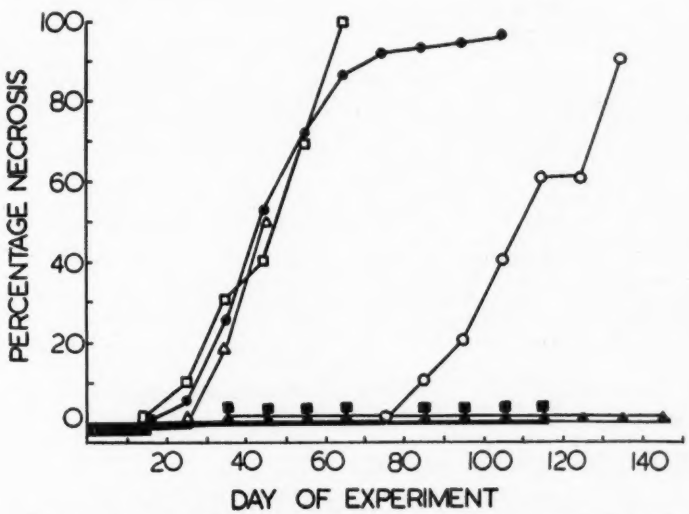


Fig. 14, B: Shows the cumulative incidence of hepatic necrosis, as percentages of the total number of animals in each of the dietary experiments specified, in relation to the duration of each experiment.

ten rats developed necrosis, the onset of the lesion was delayed. In these rats, there was a considerable amount of lobar absorption; this was usually accompanied by the accumulation of a yellow-brown pigment, probably ceroid, in the portal tracts related to the zones of necrosis.

The failure of cystine to prevent liver necrosis on our diet is at variance with what Himsworth and Glynn and Gyorgy and others have described in animals fed on their experimental diets. It must be remembered, however, that the diets used by these other investigators are much more complex than our own.

Noteworthy too is the observation that the addition of cystine to our potato starch/food yeast diet allowed rats to survive for as long as 135 days. This diet does not contain vitamin A. Even on a high protein (18 per cent), fat-free diet, lacking vitamin A, 30-40 gm. rats grow rapidly but usually die about the 70th day.

Indeed, in these circumstances, the presence or absence of vitamin A may alone decide whether low grade necrosis will supervene (unpublished data).

Since both Gyorgy *et al.* and Himsworth and Glynn have included vitamin A as well as fats in their diets, it may well be that in those contexts, cystine acquires a protective action against hepatic necrosis. It would seem, therefore, that cystine, like alpha-tocopherol, may have a sparing effect on the utilization of vitamin A. Cystine, unlike tocopherol, does not protect rats against hepatic necrosis on our diet.

(h) *Addition of alpha-tocopherol.* The administration of 1 mg. of alpha-tocopherol per day to each rat enhances the survival period, the last rat dying on the 145th day of the experiment (Fig. 14A). In this respect, alpha-tocopherol has an action similar to that of cystine. However, in contrast to cystine, alpha-tocopherol resulted in complete protection of the liver against hepatic necrosis (Fig. 14B & Table 2). In our diet, therefore, alpha-tocopherol prevents hepatic necrosis no less than it does in the diets used by Himsworth and Lindan (1949), Gyorgy *et al.* (1950) and by Gyorgy and Goldblatt (1951).

(i) *Addition of thyroid.* By speeding up the metabolism, we hoped to hasten the time of onset of hepatic necrosis. Accordingly, we fed dried thyroid (1 mg. per rat per day) to a group of rats subsisting on potato starch and food yeast. All the rats died by the 45th day and the total incidence of necrosis of the liver was 50 per cent, giving the limits of 'p' as 0.118 to 0.882 (Figs. 14A, B and Table 2).

On statistical analysis, the incidence of necrosis among these rats at the time of death was not significantly reduced as compared with that among the rats dying on potato starch and food yeast alone without dried thyroid. In the case of the latter, it will be recalled that 56 per cent of rats are dead by the 45th day of the experiment and that in the remaining 44 per cent, necrosis of the liver may not yet have declared itself (Figs. 14A & B). If all the rats on the potato starch and food yeast diet had been sacrificed on the 45th day, the incidence of necrosis might well have been the same as in those animals receiving the thyroid supplement. Contrary to expectation, the addition of thyroid to a diet of potato starch and food yeast does not accelerate the development of necrosis of the liver. Drill and Hays (1942) have shown that the liver becomes sensitive to thyroxin when the B complex content of the diet is low. In our experiment, the high vitamin B content of the food yeast may have protected the liver against the effects of thyroid. We may therefore conclude that the addition of thyroid to the potato starch and food yeast diet leads to premature death without necessarily influencing the incidence of necrosis of the liver.

TABLE 3

COMPARISON OF BREWER'S YEAST AND FOOD YEAST ON A GLUCOSE DIET IN THE PRODUCTION OF HEPATIC NECROSIS SHOWING PROTECTIVE ACTION OF BREWER'S YEAST.

Diet	Number of animals n	Number of animals with hepatic necrosis m	Incidence of hepatic necrosis m/n = p	95% limits of p
Glucose + 10% food yeast	23	16	0.696	0.471 to 0.868
Glucose + 10% Brewer's yeast	20	2	0.100	0.012 to 0.317

Substitution of brewer's yeast for food yeast in combination with glucose.

In view of the fact that investigators have not been uniformly successful in producing necrosis of the liver with diets in which the proteins were derived exclusively from the yeast, we decided to test whether brewer's yeast would be as effective as food yeast in producing liver necrosis. Accordingly, we substituted brewer's yeast for food yeast, with glucose as a source of carbohydrate. The incidence of necrosis of the liver was reduced to 10 per cent (Table 3), while the survival of the rats was not greatly altered (Fig. 15A).

Quite clearly then, the composition of the yeast can significantly influence the efficiency of the diet in producing necrosis of the liver. Gyorgy *et al.* (1950) produced a high incidence of hepatic necrosis with the yeast sent to them by Himsworth from England. They were unable to obtain the same consistently high incidence of hepatic necrosis with their own yeast.

While dismissing the difference in the methionine and cystine contents of the yeast as playing a part in modifying the incidence of necrosis, Gyorgy *et al.* were led to postulate an unidentified toxic factor in the brewer's yeast which, under the influence of the gut, promoted hepatic necrosis.

TABLE 4

COMPOSITION OF YEAST

	Cystine	*Methionine*	*Incidence of Necrosis*
Torula (Dehydrated Products Ltd.)	0.88%	0.65%	96%
Brewer's yeast (S. A. Food Extracts Ltd.)	0.45%	0.79%	20%
British yeast	0.72%	0.80%	100%
American yeast	0.43%	0.79%	50%

Even though Gyorgy *et al.* are in favour of the existence of a toxic factor, we cannot lightly overlook variations in the chemical composition of yeasts in so far as they may influence the behaviour of the liver. From a perusal of Table 4 it is apparent that the methionine content of the four yeasts listed therein ranges between 0.65 per cent and 0.8 per cent and does not provide any indication of the capacity of the yeast to produce hepatic necrosis. On the other hand, both the yeasts which are associated with a high incidence of necrosis contain almost double the amount of cystine present in yeasts associated with a low incidence of necrosis. It may well be that, within absolute limits, the ratio between methionine and cystine may determine whether or not a particular yeast will promote a high incidence of necrosis. Thus, in the torula and in the British yeast, the ratio of cystine to methionine is 1.3:1 in the former instance and 0.9:1 in the latter. In the American yeast and in our brewer's yeast, the

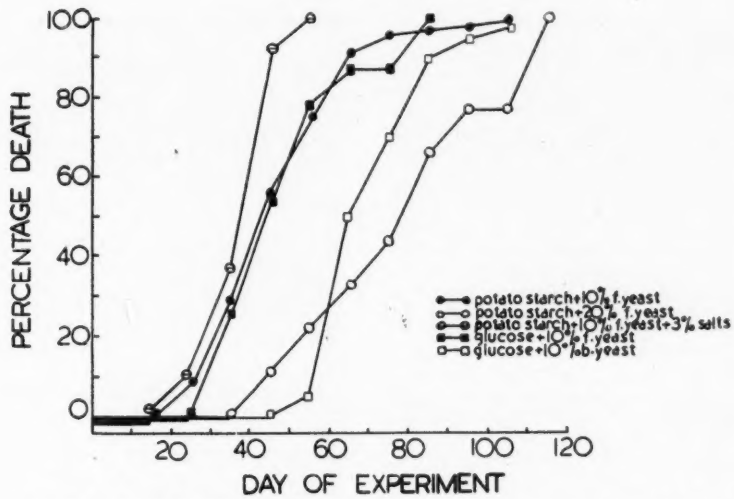


Fig. 15, A: Shows the cumulative incidence of death in relation to the duration of the experiment in rats fed the various diets specified.

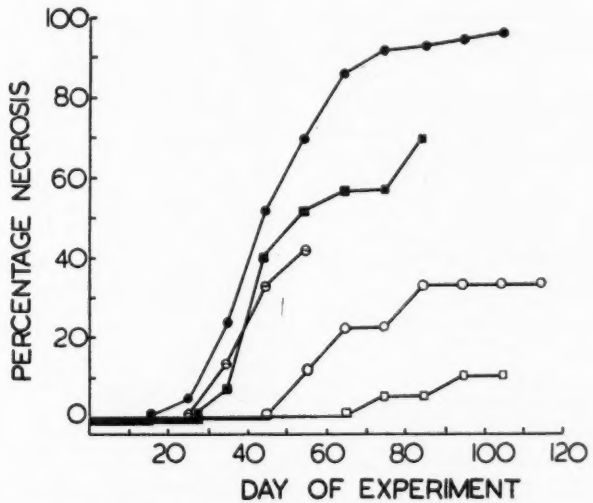


Fig. 15, B: Shows the cumulative incidence of hepatic necrosis, as percentages of the total number of rats in each dietary experiment in relation to the duration of each experiment.

ratio of cystine to methionine is 0.54:1 and 0.57:1 respectively. This is one correlation which can be established between the composition of the yeast and its necrogenic properties. It is possible that other correlations may be found. Our torula for example contains 40-50 mg.% nicotinic acid as compared with 68 mg. per cent in our brewer's yeast. These differences in vitamin content may also modify the incidence of hepatic necrosis.

Yeasts are known to vary considerably in their amino-acid and vitamin content. Different strains of yeast grown on the same medium and the same strain grown on different media can vary widely in chemical composition (Wiley *et al.*, 1950). Indeed, only when the chemical composition of yeasts is fully elucidated will it be possible to understand why one yeast is more efficient than another in producing hepatic necrosis.

COMMENT

Much of modern nutritional research is based on the assumption that the organism requires a minimum number of food factors for maintaining the integrity of its tissues and for its survival. It is believed that a deficiency of any one particular food factor is associated with a specific pathological consequence, which asserts itself either chemically or morphologically. Moreover, the pathological process is said to be prevented by the addition of adequate amounts of the missing food factor. Thus, in diets low in protein, the lack of methionine is said to be associated with the appearance of a fatty change and of necrosis in the liver. A minimum amount of methionine in the diet is said to prevent both these phenomena. This approach to nutrition has led to the elaboration of the concept of tissue specific effects of vitamins and other food factors.

Although this concept has inspired much nutritional research, with fruitful consequences, nevertheless many paradoxical situations arise for which there is no apparent explanation in terms of the above concept.

From the data presented in our nutritional experiments cited above, it is clear that as far as the behavior of the liver is concerned, the importance of methionine or vitamin E in preventing hepatic necrosis is a function of the other constituents of the diet. When young rats are fed on a diet composed exclusively of glucose or of potato starch, and therefore lacking, at least, methionine and vitamin E, necrosis of the liver does not develop. When food yeast, containing no vitamin E and low in methionine, is added in a concentration of ten per cent, either to the glucose or to the potato starch, hepatic necrosis develops in a large percentage of rats. If the prevention of necrosis was a function only of the absence of vitamin E and an insufficiency of methionine, then necrosis should have occurred in the diet consisting only of carbohydrate. Since this did not prove to be the case, it follows that the addition of food yeast was responsible for promoting a metabolism in which methionine or vitamin E, in minimal concentrations, became limiting factors in deciding whether or not hepatic necrosis would supervene.

Furthermore, the necrogenic effects of torula, when added to undextrinised potato starch, are no longer in evidence. In this latter instance, necrosis of the liver cannot be a function of the lack of vitamin E or of an insufficiency of methionine but rather of the nature of the carbohydrate. In this connection, it may also be recalled that the substitution of glucose for potato starch reduced the incidence of necrosis from 96 per cent to 70 per cent.

Similarly, by the addition of a 3 per cent salt mixture, the incidence of hepatic necrosis is also reduced.

From these several observations, we are unable to avoid the conclusion that the role of methionine or of vitamin E in preventing necrosis of the liver is not determined by the intrinsic needs of the organism for these two food factors but is dependent on the type of metabolism fostered by the experimental diet.

The rate of development of hepatic necrosis in animals on a particular diet is also profoundly influenced by the age of the animal. Thus the older the animal at the commencement of the experiment, the longer does it take for necrosis of the liver to declare itself. In rats weighing over 300 gm., the onset of necrosis is greatly delayed and the incidence may also be reduced. In this connection we would like to recall the delayed appearance of necrosis in 30 to 40 gm. rats receiving 50 mg. cystine per day in conjunction with a potato starch food yeast diet. The importance of a food factor in preventing a particular pathological process, therefore, is a function of the prevailing metabolism, the latter in turn being dependent in part on the qualitative and quantitative attributes of the various food factors in the diet and in part upon the age of the animal.

Abel and Beveridge (1949) were of the opinion that the inability of different investigators to obtain a consistently high incidence of necrosis of the liver was due, in part, to the inclusion in the experimental low protein diets of substances such as cod-liver oil or slightly rancid fats, said to inhibit the action of dietary tocopherols.

It is clear that while their explanation may apply to diets which contain cod liver oil and rancid fats, it certainly cannot account for the difference in the necrogenic effects of food yeast and of brewer's yeast in our diets which contained neither lard nor cod-liver oil. Moreover, although the addition of tocopherol prevents the onset of necrosis of the liver in our food yeast-potato starch diet as well as in our brewer's yeast diets, the absence of tocopherol from the diet cannot explain the difference in the incidence of necrosis since neither food yeast nor brewer's yeast contains detectable amounts of tocopherol. It follows that a subtle difference in the chemical composition of our yeasts and of yeasts used by other investigators influences the necrogenic properties of the diet, quite apart from the presence of cod-liver oil or slightly rancid fats.

From an analysis of our own observations as well as of the findings of other investigators, we are led to suggest that necrosis of the liver, of nutritional origin, like the fatty liver, is the end picture of disturbances in metabolism produced in several different ways. In support of this view, we may state first, that necrosis of the liver developing in rats fed a diet low in protein may be prevented by the addition of methionine or cystine (Glynn et al., 1945) or alpha-tocopherol (Schwartz, 1944, Himsworth and Lindan, 1949, Gyorgy *et al.*, 1950) or by aureomycin and other antibiotics (Gyorgy, 1951). Secondly, necrosis of the liver in rats fed on the low protein diet used by Hove *et al.* (1949) may be prevented by tocopherol but not by methionine; thirdly, that necrosis of the liver in rats fed a diet of potato starch and food yeast may be prevented by alpha-tocopherol or methionine but not by cystine; and finally, diffuse low grade necrosis, produced by high protein diets lacking vitamin A and fat but containing alpha-tocopherol can be prevented and cured by the addition of vitamin A (unpublished data). Only by careful analysis of the types of metabolism imposed by these different diets will it eventually be possible to discover the mechanisms which are immediately responsible for hepatic necrosis and why a particular dietary substance, by its presence or absence, becomes a limiting factor in preventing or promoting the necrosis.

SUMMARY

A simple dietary technique is described for the production of hepatic necrosis. The liver lesion develops

consistently in rats weighing between 30 and 200 gm. at the commencement of the experiment. The time of onset of necrosis, however, is a function of the age of the rat.

Hepatic necrosis produced by the diet is prevented by alpha-tocopherol and methionine but not by cystine or choline, although cystine delays the onset of necrosis in 30-40 gm. rats. The incidence of necrosis is also dependent upon the nature of the carbohydrate and on the presence or absence of a particular concentration of various salts.

Attention was drawn to the variation in the capacity of yeasts of different chemical composition to excite necrosis of the liver. In this connection, a suggestive correlation was established between the methionine and cystine contents of various types of yeast and their necrogenic property.

The limitations of the tissue-specificity concept of vitamin action were examined in the light of data presented in this study.

It was concluded that necrosis of the liver of nutritional origin, like the fatty liver, was the end picture of disturbances in metabolism produced in several different ways.

We are grateful to Dr. Bernard Segal of South African Food Extracts Ltd. for supplying the brewer's yeast and for the trouble he has taken in making available to us a full chemical analysis.

Our thanks are due to Mr. Charlton of Industrial Development Corporation of South Africa Ltd. and Mr. Balog of Dehydrated Products Ltd. who kindly supplied the food yeast as well as the chemical information pertaining thereto, and to Manischewitz Food Products Incorporated for providing the analysis of their potato starch.

We wish to thank Mr. Arnold Herman and Mr. Sidney Dry for their assistance in the preparation of the histological sections and photomicrographs.

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COMPLETE RESTITUTION OF THE LIVER IN RATS FOLLOWING NECROSIS AND FIBROSIS OF DIETARY ORIGIN

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WITHIN a short period after removal of one or several lobes, the liver in animals is capable of regenerating itself completely (Fishback, 1929, Herxheimer and Thölldte, 1930). Even when large parts of the liver are destroyed by toxic substances, whether of chemical or bacterial origin, complete restitution has been recorded both in animals (Klotz and Belt, 1930) and in human beings (Dible, McMichael and Sherlock, 1943, Lucké, 1944). Moreover, when animals are subjected at irregular intervals to the influences of carbon tetrachloride (Whipple and Sperry, 1909) or of butter yellow (Orr, 1940), the livers are not only damaged but undergo a considerable degree of fibrosis.

Although a human liver injured by chemical agents or by the virus of infective hepatitis may regenerate completely, nevertheless connective tissue overgrowth may occur, leading to hepatic fibrosis (Bergstrand, 1930, Cullinan, 1936, Krarup and Roholm, 1941, Lucké, 1944, Dible, McMichael and Sherlock, 1943, Watson and Hoffbauer, 1946). The reasons usually given for the development of hepatic fibrosis are: (1) destruction of the framework of the liver lobule (Himsworth and Glynn, 1944, a & b, Glynn & Himsworth, 1944), (2) altered blood supply to the liver (Mann and Bollman, 1938) and (3) the persistence of the nocuous stimulus

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with repeated injury to the liver parenchyma (Lucké, 1944).

In man when it occurs restitution may be achieved in 1-14 months (Lucké, 1944). If the administration of carbon tetrachloride or butter yellow to rats be discontinued, not only may the fibrous tissue disappear but also through regeneration, the normal liver architecture may be restored (Cameron and Karunaratne, 1936, Steinberg and Martin, 1946).

Acute liver injury induced by diet may also undergo complete resolution, particularly in the case of the fatty liver both in man (Gillman & Gillman, 1951) and in animals (Best & Lucas, 1943, Lowry, Ashburn and Sebrell, 1945). Experimentally it has been shown that after the supervention of hepatic fibrosis of nutritional origin a change of diet may only arrest the progress of the fibrosis but apparently does not promote the removal of the fibrous tissue already present (Lowry *et al.*, 1945).

The object of the present study is to demonstrate (1) that in appropriate circumstances acute, diffuse necrosis of the liver of dietary origin in rats can be followed by complete regeneration, (2) that, even where fibrosis supervenes after acute necrosis of dietary origin, restitution of liver structure can also be achieved, (3) that repeated injury to the liver induced by diet may be attended by gross distortion of lobar architecture. From a consideration of the relevant experimental findings we shall comment on the relationship of necrosis to fibrosis in so far as it may disclose the role of diet in promoting the wide range of morphologies observed in chronically malnourished communities and in cases of infective hepatitis.

METHODS

In a previous communication (Gillman et al., 1952) we showed that necrosis of the liver can be produced consistently in rats by a simple dietary technique. For the purpose of our present study, 30-40 gm. rats were fed the experimental diet until it was suspected, on clinical grounds, that liver injury might have supervened. At this stage, the rats were laparotomised under ether anaesthesia. In those animals where the liver displayed extensive damage, a wedge of the most severely injured portion was excised for histological examination. After operation, the rats were gradually given access to a full diet, such as is given to our stock animals. After a variable period of time, the animals were sacrificed and sections of the liver were removed from the same lobe from which the first biopsy was taken. It thus became possible to compare the reactions of the liver at the time of the onset of necrosis and after the acute reaction had subsided.

RESULTS

Restitution of the liver following acute massive hepatic necrosis of dietary origin.

Figs. 1 & 2 are sections of the liver obtained by laparotomy of a rat following a period of 65 days on a diet of potato starch and food yeast. The extent of the acute hemorrhagic massive necrosis is clearly evident. Entire lobules, including the connective tissue framework from central vein to portal tract, are completely destroyed, the bile ducts are moderately distended (Fig. 2), the portal vein greatly dilated and numerous round cells are scattered in the portal tracts. Immediately after the biopsy was taken the animal was transferred from the necrogenic to an adequate diet such as is fed to our stock rats.

At the end of 300 days on a stock diet, the rat was sacrificed. At autopsy all the lobes of the liver, including the one from which the biopsy had been taken were smooth and well formed, and to the naked eye, disclosed no obvious pathology. The only exceptional feature was the scalloping of the free edge of the liver and the slight dilatation of the related veins.

On histological examination, all lobes of the liver presented an entirely normal appearance; indeed, were it not for the presence of a few pigmented cells in the portal tracts, which we know to be associated with previous injury to the liver, the section shown in Fig. 3 could quite easily be regarded as coming from that of a normal animal. It would be difficult to believe that the portion of the liver in Fig. 3 was removed from the same lobe showing the extensive destruction portrayed in Figs. 1 and 2.

Even when the liver cells and the framework of the liver are completely destroyed over large areas, the surviving cells can proliferate and restore completely the architecture of the lobules, provided an adequate diet is available.

Recovery of the liver following hepatic fibrosis and lobular distortion.

We have established previously that the livers of some animals on a potato starch and food yeast diet may undergo partial but extensive necrosis, to be followed later by the development of post-necrotic scarring. Accordingly, we laparotomised a series of rats which had been on the necrogenic diet for a variable period of time. Biopsies were taken from the most distorted lobes. After operation the animals were transferred to a full diet.

Figs. 4 and 5, sections taken at laparotomy from the injured lobe of the liver, displayed all the features characteristic of post-necrotic scarring. When stained with haematoxylin and eosin, gross distortion of the liver structure is at once apparent. The depressed portion of the free surface is seen to be continuous with large mass of fibro-cellular tissue in the liver substance; nodular hyperplasia is evident and the usual topographical relationship between the central vein and the portal vein is now absent. In a reticulum preparation (Fig. 5) the gross lobular distortion is still further emphasized and a dense meshwork of fibres extends from the free surface into the substance of the liver. Such an increase of fibrous tissue is grossly abnormal for the rat and can be taken to mean that hepatic fibrosis has supervened.

Fig. 6 is a section from the same lobe of the liver as displayed in Fig. 4, 300 days after the transfer of the rat from the potato starch and food yeast diet to a full diet. Apart from the vacuolated glycogen-rich liver cells and the cellular portal tract, the architecture of the liver is restored to an almost normal condition. The well developed bands of fibrous tissue observed in the liver in the first biopsy specimen (Fig. 5) had completely disappeared, leaving behind the delicate reticulum framework (Fig. 7) now indistinguishable from that seen in normal animals. The silver precipitate in the liver cells in Fig. 7 is due to the great accumulation of glycogen.

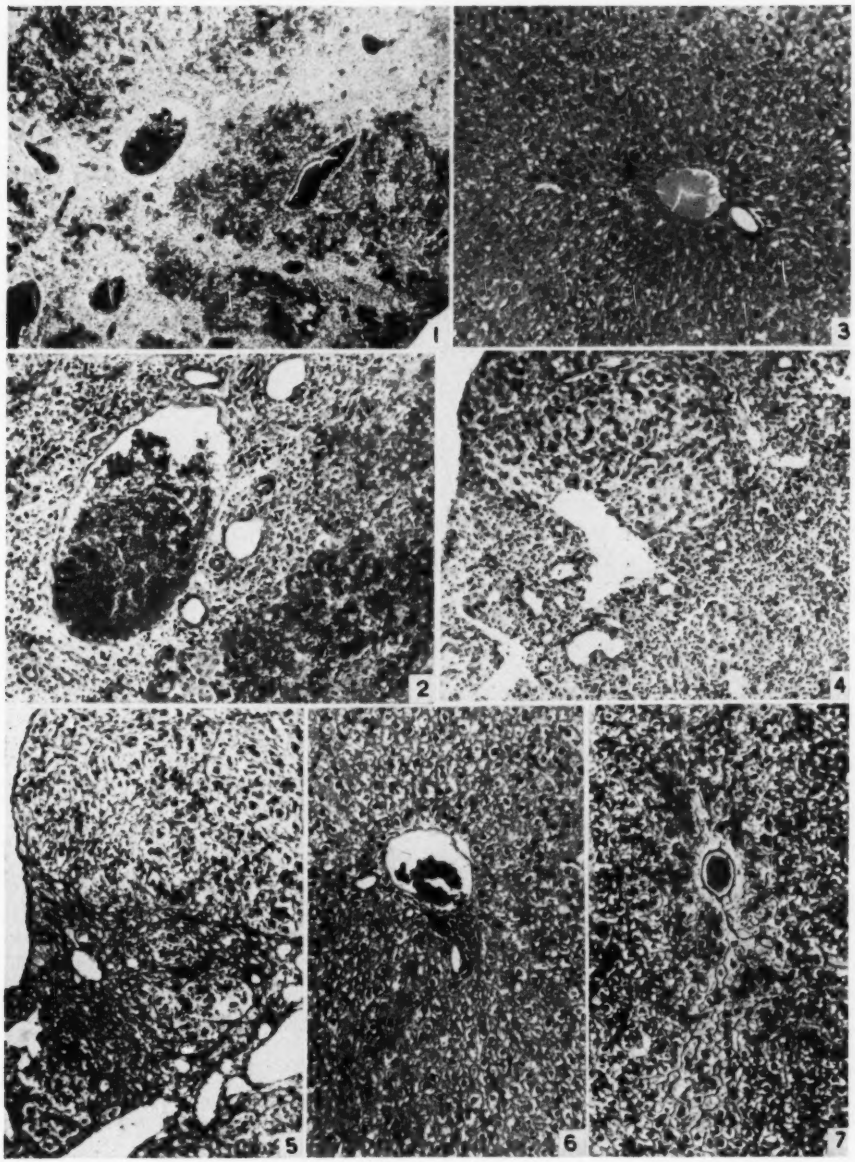


Fig. 1: Massive necrosis of the liver as revealed in a biopsy specimen at the end of 65 days on a diet of potato starch and food yeast.

Fig. 2: High power study of the portal tract depicted in Fig. 1.

Fig. 3: Section of the same lobe of the liver as in Figs. 1 & 2 taken after 300 days on stock diet. Note complete restitution of liver structure.

Fig. 4: Biopsy of liver of rat after 65 days on a diet of potato starch and food yeast. Note distortion of liver architecture, the approximation of the hepatic vein and the portal tracts, the great increase in fibroblastic cells and nodular hyperplasia.

Fig. 5: Same liver as shown in Fig. 4 impregnated with silver (Wilder's technique). Note the dense mass of fibres extending from the depressed surface of the liver (post-necrotic scarring).

Fig. 6: Section through the same lobe of the liver as Figs. 4 & 5 after 65 days on stock diet. Note the slight cellularity of the portal tract and restitution of liver architecture.

Fig. 7: Same liver as in Fig. 6 impregnated with silver to demonstrate reticular fibres. The framework of the portal tract and of the trabeculae has almost returned to normal.

We can therefore conclude that the overgrowth of fibrous tissue developing in a liver after suffering extreme necrosis of dietary origin, is not irreversible. Provided the diet is corrected, even large bands of fibrous tissue may disappear completely and the original structural organization of the liver lobule may be completely restored.

The response of the livers of rats fed alternately with potato starch and food yeast and with stock diet.

A group of twenty rats weighing 30-40 gm. was fed a diet of potato starch and food yeast for one month. They were then transferred to a stock diet for 3 days and thereafter returned to the necrogenic diet. At the end of the second month the rats were again transferred to a stock diet for a short period of 3 days and then returned to the diet of potato starch and food yeast. This procedure of alternating the diets was repeated at regular intervals. After subjecting the animals to this regimen for 4 months, the period during which a stock diet was fed was increased to 7 days in order to allow the animals to gain weight. Although some animals died during the progress of the experiment, the majority survived for well over 300 days. At autopsy, the livers displayed a variety of morphological pictures ranging from acute necrosis to gross distortion of the lobes, the latter simulating Laennec's hepatic fibrosis described in man.

Histologically, a liver can show an area of extensive necrosis of relatively recent origin with commencing fibrosis and nodular hyperplasia (Fig. 8). Sometimes, on histological section, bands of fibrous tissue can be seen breaking up the liver into numerous hyperplastic nodules associated with a profound change in the arrangement of the vascular channels (Fig. 9).

In some livers the remains of old necrosis were represented as fine fibrocellular bands extending from the depressed surface of the liver towards some of the deeply-situated portal tracts (Fig. 10). Such a reaction in our experimental animals is usually associated with extensive nodular hyperplasia.

It is noteworthy that, although several livers which to outward appearance displayed gross distortion of the surface of the lobes, only a moderate increase in fibrous tissue with marked enlargement of the lobule may be detected on microscopic section. In man Laennec's fibrosis can often be more easily recognised with the naked eye than under the microscope (Mallory, 1945 and Patek, 1945). A similar phenomenon also seems to be a feature of the rat. Moreover, it is perfectly clear from our sections of rat livers that the arrangement of the blood vessels has been profoundly modified. Indeed it seems to us that the peculiar distribution of the vessels and the hypertrophy of the related lobules rather than the fibrosis are responsible for the distortion of the liver seen with the naked eye.

From this experiment it is evident that, although the survival of animals fed a necrogenic diet can be prolonged by occasional feeding for limited periods with stock diet, the liver remains vulnerable, and can still be grossly disorganised.

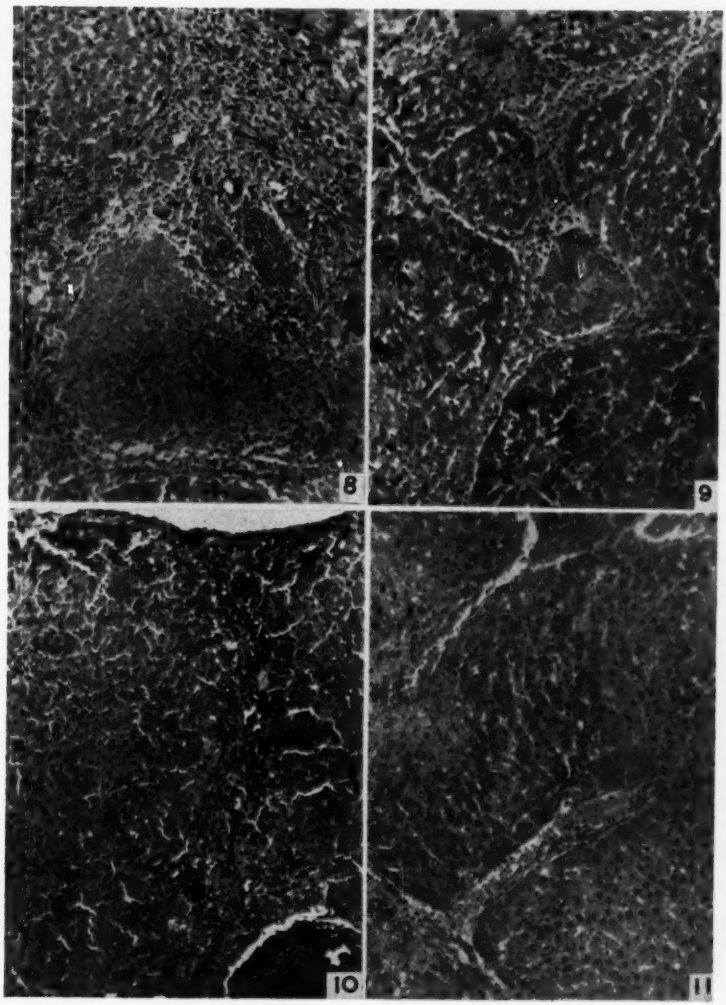
Although acute lesion still occurred, there was a tendency for liver damage to declare itself in a more chronic form. Clearly this more chronic reaction can be attributed to the prolonged subsistence of animals on a bad diet, punctuated by short periods of exposure to the effects of a good diet. Noteworthy, too, was the great individual variation in the reactions of the livers of rats fed this dietary regimen. The difference in reactivity may be attributed to differences in the ability of rats to take advantage of the good diet, in turn determined by the extent of pre-existing liver and other organ damage imposed by the necrogenic diet.

COMMENT

Our nutritional experiments in rats disclose that extensive necrosis of the liver, involving entire lobules together with their supporting framework of reticular fibers, need not be attended by an overgrowth of fibrous tissue characteristic of post-necrotic scarring. Provided the correct dietary conditions are satisfied, despite extensive necrosis, the liver architecture can be so completely restored that it may be extremely difficult to detect any evidence of previous gross injury. On the other hand, once rats have developed extensive hepatic necrosis, provided the rats are maintained on the necrogenic diet, post-necrotic scarring is then the usual sequel.

The serial development of hepatic necrosis and then of fibrosis has led to the widely accepted belief that post-necrotic scarring or hepatic fibrosis is the automatic consequence of hepatic necrosis. It is true that as long as rats subsist on the necrogenic diet hepatic fibrosis seems to be coupled immediately with the antecedent necrosis and the disruption of the connective tissue framework of the liver lobule. However, we have shown that once hepatic necrosis has supervened the overgrowth of fibrous tissue or complete regeneration is not a function of the extent of disruption of the hepatic lobule but rather of the nature of the diet fed the experimental animal. By appropriate manipulation of the necrogenic and of the adequate diet we have been able to disengage necrosis from fibrosis and hence promoted regeneration instead of post-necrotic scarring. Quite clearly the mechanism responsible for necrosis is not identical with that favouring fibrosis. Both phenomena require special and perhaps different metabolic conditions for their emergence. Where necrosis has supervened and provided the metabolism is favourable, only then will necrosis be attended by fibroblastic activity. The products of liver degeneration in a particular metabolic context may stimulate an overgrowth of fibrous tissue and exclude a harmonious regeneration of liver cells and of the associated vascular and reticular framework.

Fibrous tissue overgrowth in an organ like the liver is usually regarded as an irreversible phenomenon. Its persistence is almost included as an "automatic" affair having little relation to the local or general metabolic conditions within the organism and this despite several suggestive observations to the contrary. Thus, in the early stages the fibrous tissue response elicited in rats treated chronically with carbon tetrachloride or with butter yellow will not only be maintained but will progress as long as these toxic substances are administered. Once these toxic substances are withheld, the fibrous tissue may disappear completely from the liver. The disturbed metabolism provoked by the toxins promotes the growth and persistence of the fibrous tissue and is immediately dependent on the presence of the toxins. Similarly, the persistence of the fibrosis following necrosis of the liver in our rats is conditional upon the



Figs. 8, 9, 10, 11 are sections of livers of rats subsisting alternately on the potato starch-food yeast diet and for short periods on stock diet (see text).

Fig. 8: Shows two stages in extensive necrosis of the liver with nodular hyperplasia.

Fig. 9: Fibrous bands demarcate the liver into irregular lobules. Macroscopically this liver resembled a Laennec's fibrosis.

Fig. 10: Low grade hepatic necrosis with enormous enlargement of the related lobules. Macroscopically the liver showed irregular scarring.

Fig. 11: Large irregular lobules are separated by relatively thin bands of fibrous tissue but macroscopically the liver presented a coarse, nodular surface.

continued feeding of a particular diet. The diet assumes a role analogous to that of the toxic substances and maintains the fibrous tissue promoting metabolism. By changing the diet, the metabolism is altered and, as in the case of chronic carbon tetrachloride poisoning, overgrowth of fibrous tissue of nutritional origin in the liver can be arrested and, in appropriate circumstances, may be permitted to disappear completely.

Disappearance of the fibrous tissue and regeneration of the liver is a slow process and will only occur after the animal has been transferred to a good diet for a minimum period of time. The healing of the liver is

also governed in part by the extent of the hepatic disorganization. If a complete lobe is destroyed, it may not be replaced but other lobes may hypertrophy as a compensatory measure.

As already indicated, alternate exposure to the necrogenic diet and to the stock diet leads to profound modification in liver structure. While subsisting on a bad diet, necrosis and often fibrosis as well are generally observed; during the short period on the stock diet, the surviving lobules may enlarge considerably with some reduction in the amount of fibrous tissue. If regeneration has not been completed when the animal is returned to the bad diet, necrosis may once again supervene. By manipulating the time of exposure to the good and to the bad diet, it is possible to obtain a diversity of liver pathologies. Experiments of this nature suggest some of the possible factors at play in producing the wide range of hepatic disease encountered in malnourished communities. Prolonged exposure to a bad diet, punctuated by periods of greater or lesser duration when protective nutrients in varied amounts become available to the community, may lead to a wide range of liver pathology analogous to that which we have described in our experimental rats.

The question still remains unanswered as to why the livers of seemingly well-nourished individuals after an attack of infective hepatitis may restitute completely in some and may progress to fibrosis in others. As shown above in the case of nutritional experiments, restitution of liver structure and fibrosis is made possible by specific types of metabolism and is not necessarily dependent upon the extent of the disorganization of the liver lobule and of its supporting reticular network. The fact that two individuals with comparable degrees of liver injury may react so divergently to the same infection implies a profound difference in the individual metabolism at the time the infection was contracted. The virus merely exposed the underlying difference in metabolism in these two subjects. Even if such individuals were consuming similar diets, the nutrients therein could not be utilized in an identical manner.

In this connection we may recall the classical experiments of Chaikoff and his associates (Chaikoff et al., 1938, 1948, Gillman & Chaikoff, 1949) who demonstrated the altered utilisation of food in thyroidectomised, hypophysectomised and pancreatectomised dogs. Fatty livers developed in all these animals while consuming a diet rich in lipotropes and adequate to prevent a fatty liver when fed to normal dogs. However, the fatty liver of the dogs with one or other of the endocrine glands ablated could be prevented by increasing the choline in amounts which ordinarily would not be available in a good diet.

By analogy, human subjects suffering from varying degrees of hypothyroidism or of pancreatic disease will not utilise the same food in the same way as another individual with a normally functioning thyroid or pancreas. Indeed, as revealed in the case of hypothyroidism, nutritional diseases may even supervene on a diet adequate to maintain an intact organism in good health (Gillman & Gillman, 1951). If then, two individuals, one in good health, the other suffering from subclinical hypothyroidism, are both consuming the same diet, in the one case the diet will be adequate and in the other grossly deficient. If these two individuals both develop an attack of infective hepatitis, divergences in hepatic reactions are to be expected, in the same way as has been shown in the case of the reaction of livers of animals subsisting on different diets and poisoned with the same concentration of chloroform. The divergent reaction of livers to the same stimuli, therefore, is dependent on the prevailing metabolism. The latter is not only a function of the diet consumed but also of the way the nutrients are utilized. The utilization of food in turn is dependent on the functional integrity of the various organs and tissues as we have shown to be the case in chronic malnutrition in man and as has been described experimentally in animals.

SUMMARY

Both acute necrosis and fibrosis of the liver of dietary origin in rats can be followed by complete restitution of structure in an appropriate dietary setting.

Necrosis will only be followed by hepatic fibrosis in special dietary circumstances.

By manipulation of the diet, a wide diversity of liver pathologies has been produced, analogous to that seen in malnourished communities.

An explanation has been offered to account for the differences in reactivity of the liver to nocuous stimuli such as the virus of infective hepatitis. In this connection, the relationship of the diet, its utilization and the integrity of the tissues of the organism to liver necrosis has been emphasized.

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THE INSULIN GLUCOSE TOLERANCE TEST AND THE ABSOLUTE LYMPHOCYTE RESPONSE IN DIABETIC PATIENTS

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THE REGULATION of the blood glucose level has been shown to depend on the activity of the liver. Thus, when the blood sugar level is raised the liver responds by decreasing its glucose output and, on the other hand, a fall in the sugar level is compensated by an increased release of hepatic glucose. The ability of the liver to respond to changes in the blood glucose level depends in turn on a normal balance between the action of insulin and the hormones of the anterior lobe of the pituitary and of the adrenal cortex (1).

In the normal individual glucose administered either orally (2,3) or intravenously (4) is accompanied by a significant decline of the absolute lymphocyte count. This decline does not occur in the adrenalectomized animal or in the Addisonian patient, and is thought, therefore, to be mediated by activation of the pituitary adrenal axis caused by the change in the blood sugar level (5). However, when glucose is administered to the diabetic patient it is found that approximately 50 percent do not show the expected decline of the absolute lymphocyte count (6) and this group of patients is thought, therefore, to have some defect in the pituitary adrenal mechanism.

On the other hand, the intravenous injection of small doses of crystalline insulin to the normal individual evokes a marked rise of the absolute lymphocyte count (4). The exact mechanism for this phenomenon is not known, but since in a series of diabetic patients the mean percentage rise of the absolute lymphocyte count declined with increasing degrees of abnormality of the insulin tolerance curve, it is thought that hypoglycemia responsiveness and the rise of the lymphocyte level are similarly mediated (6).

In 1949 Engle and Scott (7) introduced the insulin glucose tolerance test (I.G.T.T.) as an improved procedure for the detection of hypoglycemia unresponsiveness in Addisonian and hypopituitary patients. They felt that on the basis of previous experimental evidence presented by Somogyi (8), glucose magnifies the normal homeostatic response to hypoglycemia. It was suggested (7) that the prompt elevation of the blood sugar in the I.G.T.T. after ingestion of glucose depends on 1) a rate of utilization of carbohydrate which is less than the rate of supply of glucose from the gastro-intestinal tract and from the liver by glycogenolysis and gluconeogenesis, 2) a normal ability to absorb glucose from the gastro-intestinal tract, 3) an adequate level of liver glycogen, and 4) a normal ability to release glucose from the liver. These factors are in turn to a large extent dependent on the integrity of the endocrine mechanisms for blood sugar homeostasis.

However, in a previous report we demonstrated (9) that although the rise of the blood sugar level in the I.G.T.T. above the lowest value is greater than that which would be observed in the ordinary Glucose Tolerance Test (G.T.T.) the maximal rise above the fasting value is of the same magnitude as that in the G.T.T. Moreover, a study of the variations of both the serum glucose and absolute lymphocyte level in this procedure in the normal individual indicated that the response obtained represented a summation of the effects of the independent administration of glucose and insulin. On the other hand, since this procedure activates in rapid succession the mechanisms for both hypo- and hyperglycemia responsiveness, it probably thereby places them under stress, and might, therefore, be used in the elucidation of defects of blood sugar homeostasis. These considerations led to a study to evaluate any differences of blood sugar or absolute lymphocyte pattern which might exist in the diabetic patient subjected to the I.G.T.T. as compared to the normal individual.

MATERIAL AND METHODS

On 20 non-diabetic individuals and 25 known diabetic patients an intravenous G.T.T. and I.G.T.T.

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were performed. In addition, six normal persons of average weight and nutritional status received normal saline solution intravenously instead of the glucose and insulin.

For the G.T.T. 25 Gm of glucose in a 50 percent solution were administered intravenously and capillary blood samples were withdrawn immediately before and at 30, 60, 120 and 150 minutes after the glucose.

For the I.G.T.T. 0.1 unit of crystalline insulin per kilo of body weight was given intravenously followed 30 minutes later by 0.8 gm of glucose per kilo orally. Immediately before and at 20, 30, 45, 60, 90, 120 and 150 minutes after the insulin administration, duplicate capillary blood samples were withdrawn for glucose determination and for total leucocyte counts and three smears for differential counts were made.

The blood sugar was determined by the Nelson modification of the Folin Wu Micro-Method (10). The estimation of the absolute lymphocyte value was done as suggested by Elmadjian and Pincus (11) except that the total lymphocyte count was done on two separate specimens, both sides of the counting chamber being used each time. The differential counts were performed on 200 cells on each of three smears. In order to minimize personal error, all counts were done by the same technician. The absolute lymphocyte value was obtained by multiplying the average value for the total leucocyte count by the average percentage of lymphocytes. In conformity with the method of reporting results utilized by others, the absolute lymphocyte value was expressed in terms of the percent change from the fasting value which was taken as zero percent (12, 13).

The standard deviation for this method was calculated to be ± 7 percent (14, 15). In 28 determinations of the absolute lymphocyte count in the six controls who received normal saline intravenously, the values obtained ranged from +15 to —14.9% of the initial level. These figures compared favorably with the expected chance error of ±21% or three times the standard deviation. Therefore, in evaluating the experimental series, any changes of the absolute lymphocyte count of ±21% or less of the fasting value were considered to be non-significant.

RESULTS

The criteria for a normal G.T.T. were a fasting glucose level below 140 mg% with a return of the level to or below fasting at or before 120 minutes after glucose administration (16). All the control in-

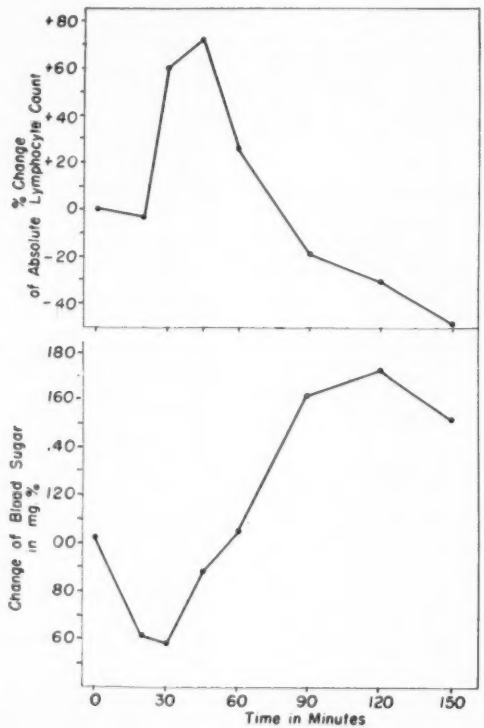

Fig. 1: Mean change of blood sugar level and of absolute lymphocyte count in 20 non-diabetic individuals during the I.G.T.T.

dividuals and none of the diabetic patients satisfied both these criteria. In two patients with mild disturbance of carbohydrate metabolism the fasting level was below 140 mg% but the blood sugar at 120 minutes was slightly above the fasting value. They were included in the series, however, since on previous occasions markedly abnormal glucose tolerance curves had been obtained in them, which justified a diagnosis of diabetes mellitus.

All of the 20 non-diabetic persons in whom the I.G.T.T. was performed showed an initial decline of the blood sugar level varying from 38 to 61 mg% below the fasting value with a mean decline of 52 mg%. The nadir occurred at 20 minutes in 8 individuals and at 30 minutes in 12. The blood sugar then rose so that at 45 minutes it was 20 to 58 mg% (average 37 mg%) above the lowest value and had again reached the fasting level by the 45 minute specimen in 5 individuals, by the 60 minute specimen in 11, and by the 90 minute specimen in 4. The maximum blood sugar value was attained at 60 minutes in 1 person, at 90 minutes in 6, at 120 minutes in 9, and 150 minutes in 4. This maximum value ranged between 132 and 195 mg% with a mean of 169 mg%. Its height above the nadir varied from 67 to 141 mg%, averaging 118 mg%, while its height above the fasting level varied from 43 to 96 mg% with a mean of 74 mg% (Fig. 1).

In the non-diabetic persons the absolute lymphocyte value after a non-significant initial decline showed a marked rise to a maximum of +41 to +178% of the fasting value with a mean of +73% which was attained at 30 minutes in 10 persons, at 45 minutes in 8, and at 60 minutes in 2. This was followed by a decline which reached a low in all persons at 150 minutes. The minimal values varied from —27 to —68% of the fasting value with a mean of —49% (Fig. 1).

In the 27 diabetic patients the initial decline of the blood sugar ranged from 9 to 98 mg%; the nadir occurred at 20 minutes in 1, at 30 minutes in 15, at 45 minutes in 10, and at 60 minutes in 1. In 4 of the 16 patients in whom the nadir was reached before 45 minutes, the blood sugar rose less than 5 mg% up to that time, which is significantly less than the rise observed in the normal. In the other 12 of these 16 patients, the 45 minute specimen was 15 to 48 mg% higher than the minimal value. The fasting blood glucose level was again reached within 45 minutes in 4 instances, within 60 minutes in 11, within 90 minutes in 11, and within 120 minutes in 1. The

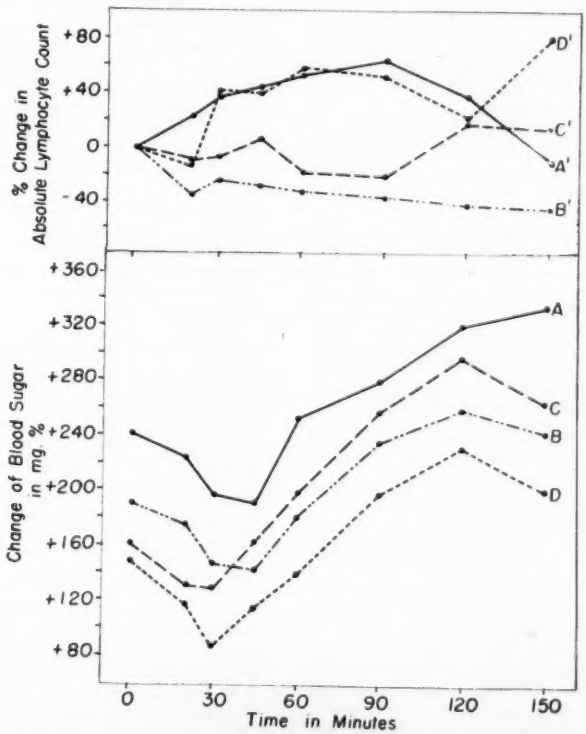

Fig. 2: Patterns of the I.G.T.T. and the change of absolute lymphocyte count in 4 diabetic patients representing the types of response observed in a series of 25 diabetic patients.

maximum blood sugar value which varied from 185 to 480 mg% (average 283 mg%) was reached at 90 minutes in 3 patients, 120 minutes in 11, and at 150 minutes in 13. This value varied from 108 to 233 mg% (average 150 mg%) above the nadir, and 42 to 213 mg% (average 111 mg%) above the fasting level. The extent or timing of the blood sugar response of the diabetic patients had no apparent relationship to the fasting blood sugar or the severity of the diabetes.

In 12 of the 27 diabetic patients the pattern of the lymphocyte response was similar to that of the controls. However, either the initial rise above the fasting level or the secondary decline was significantly less than that observed in the normal. In eight patients a significant initial rise was noted, but the secondary decline did not occur. Five persons showed no initial rise but a decline instead. In 2 patients there was a non-significant fluctuation of the lymphocyte value about the fasting level. The type of lymphocyte response did not seem to be determined by the character of the blood sugar response, by the initial blood sugar level, or by the severity of the diabetes. Examples of the blood sugar patterns obtained in diabetic persons after the I.G.T.T. and of each of the four types of lymphocyte response are demonstrated in Fig. 2.

DISCUSSION

The criteria advanced by Engel and Scott (7) for a normal blood glucose response to the I.G.T.T. were the characteristic diphasic pattern of the curve with a low within 30 minutes and a return of the blood sugar level to fasting within 30 or 60 minutes after the glucose administration (90 minutes after insulin). All non-diabetic individuals of our series conformed to these criteria and, in addition, showed a 45 minute level which was significantly above the nadir, and had a maximal blood sugar value of 195 mg% or less. Therefore, our criteria for impairment of carbohydrate homeostasis using the changes of blood sugar level in the I.G.T.T. were considered to be either 1) a failure of the 45 minute specimen to rise significantly over the nadir, 2) a failure of the blood sugar level to return to fasting within 60 minutes after glucose administration, or 3) a maximal blood sugar level of 200 mg% or higher.

None of the diabetic patients showed blood sugar curves which conformed to all three criteria. In 15 patients the lowest blood sugar level occurred at 45 minutes rather than at 20 or 30 minutes, or the 45 minute level was only 5 mg% or less greater than the nadir. This would seem to indicate a defect in hypoglycemia responsiveness in these diabetic patients. The fasting blood sugar level did not seem to bear any relationship to this type of response, which is in accord with our previous observations on the Insulin Tolerance Test (I.T.T.) in diabetics (6), in whom it was noted that the degree of impairment of the I.T.T. had no relationship to the blood sugar level, many patients showing evidence of counter-regulation of a falling blood sugar even when the serum glucose was above 100 mg%. This does not bear out reports of other investigators (8, 17) that the height of the fasting blood sugar will determine the type and time of response to a standard dose of insulin. The other 12 diabetic patients whose I.G.T.T. indicated normal responsiveness to a falling blood sugar level all showed a maximum value of greater than 200 mg%. These findings are particularly noteworthy since, as previously stated, 2 of our diabetic patients had a glucose tolerance curve which showed only very minor deviations from the accepted normal criteria.

All non-diabetic individuals showed a uniform pattern of lymphocytic response to the I.G.T.T. (Fig. 1) with an initial rise of +41% or greater, and a secondary decline to at least —27%. These limiting values together with the shape of the curve are taken, therefore, as the criteria for a normal lymphocyte response to the I.G.T.T.

No diabetic patient showed a curve which fulfilled these criteria. Even in those patients in whom the pattern approached that of the non-diabetic person, either the initial increase of the lymphocyte level or the decline following the rise was significantly less than that observed in the non-diabetic persons (Fig. 2). An attempt to correlate the pattern of lymphocytic response to the I.G.T.T. with the severity of the diabetic syndrome was unsuccessful. Neither did the disease associated with the diabetic condition seem to be a significant factor in determining the lymphocytic response to the I.G.T.T.

In most cases of diabetes mellitus, the routine G.T.T. is a satisfactory procedure for establishing the presence of impaired carbohydrate tolerance. However, in some individuals in whom the two hour blood sugar level of the G.T.T. may be at or close to the fasting value, the diagnosis of diabetes mellitus may be questionable. This is seen particularly in doubtful cases in whom mild abnormalities of carbohydrate metabolism may persist with little progression for many years. From a prognostic viewpoint, an adjuvant method of helping to identify the presence or absence of diabetes is of considerable importance. From the results obtained in this study it is believed that the I.G.T.T. either alone or in conjunction with the absolute lymphocyte response may serve as such an auxiliary procedure.

The I.G.T.T. which in rapid succession places marked stress on the homeostatic mechanisms for hypo- and hyperglycemia responsiveness apparently will show abnormalities even in cases in whom the G.T.T. is of doubtful significance. In addition, since the lymphocyte response to changes in the blood sugar level is also in part presumably mediated by the same mechanisms which control blood sugar homeostasis, the abnormalities of the lymphocyte curve may also aid in differentiating the diabetic from the non-diabetic subject.

SUMMARY

The intravenous administration of 0.1 unit of crystalline insulin per kilo of body weight followed 30 minutes later by oral administration of 0.8 gm of glucose per kilo in 20 non-diabetic individuals produced a characteristic diphasic blood sugar curve with 1) a low within 30 minutes and a 45 minute level or 20—58 mg% above the nadir, 2) a return to the fasting value within 90 minutes after the insulin, and 3) a maximum blood sugar level of less than 195 mg%. This was accompanied in all cases by a rise of the

absolute lymphocyte count to 41% or more above the fasting value followed by a secondary decline to at least 27% below the initial level.

None of the 27 diabetic patients fulfilled all three criteria for a normal I.G.T.T. In addition, the lymphocyte response to the I.G.T.T. differed in each case in some particular from the type of response observed in the normal.

It is suggested, therefore, that the I.G.T.T. either alone or in conjunction with the absolute lymphocyte response might be used as an adjuvant method for the diagnosis of diabetes mellitus in those cases where the interpretation of the G.T.T. is doubtful.

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ON THE POSSIBILITY OF REPRODUCING THE CEPHALIC PHASE OF GASTRIC SECRETION

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IT IS AN established fact that the results of a test meal may undergo marked variations from day to day, but as shown by several workers the secretory activity of the stomach may be reproduced by repeated examinations carried out by a number of different methods. By fractionated collection of oatmeal gruel Bell and MacAdam (1) obtained rather uniform secretion curves in the same subject on 20 different days. Following alcohol stimulation Bloomfield and Keefer (2) found the gastric secretion to be of fairly constant acidity and volume under standard conditions. Others (3, 4, 5) have reported the same findings in histamine experiments.

In a previous paper (6) the writer reported a sham feeding test which appeared to afford a clear picture of the cephalic secretion and which moreover gave approximately the same result in a few subjects tested on different days. Since, however, a concept as intangible as appetite must be presumed to be an important factor in deciding the secretory result of the sham feeding test, it seemed interesting to conduct a more extensive study of how far the cephalic phase may be reproduced from day to day.

Method and Material. The sham feeding test mentioned above (6) was employed in a total of 23 subjects, each of whom was tested twice at an interval of 1 to 3 days. Each of the samples collected was tested for volume of secretion, acidity (Congo red and phenolphthalein), total chloride, and pepsin by the method of Mett, and moreover the admixture of bile was estimated.

Results. The maximum values obtained in each experiment are given in Table 1. Figs. 1-4 set out 4 double experiments on 4 normal subjects performed on two different days.

DISCUSSION

A glance at the first five double columns shows quite good conformity between the majority of the corres-

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Submitted Jan. 26, 1952.

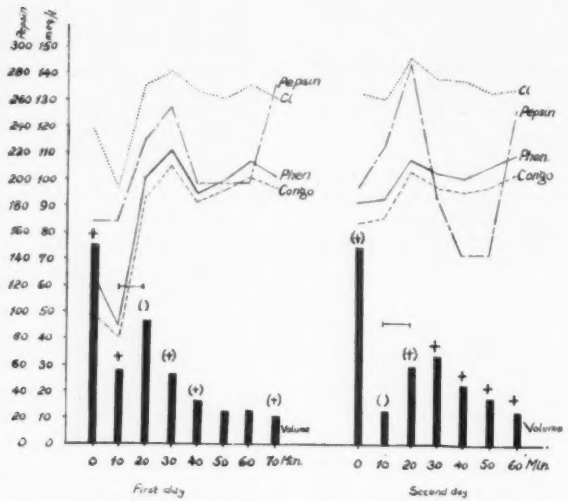

Fig. 1: Sham feeding test on the same subject with an interval of a few days. At 0 the fasting residuum. Sham feeding, marked by a short line, continued from the 10th to the 20th minute. (), (+), and + admixture of bile.

ponding maximum values. As pointed out by Flensborg (5), however, an estimate of the standard deviation to be expected at a repeated test may be based on such sets of observations, since the series of poor estimates of the standard deviation afforded by the two sets of observations may make up one good estimate owing to the additive principle in the distribution of the standard deviation squares.

Column 6 (Table 1) gives the mean of the phenolphthalein values found on two different days and Column 7 the sum of the squares on the deviation of the two observations from the mean. From these sums of squares, the mean square is computed as 71.65 (with 23 degrees of freedom). In the last column the X^2 values are calculated by dividing the individual sums of squares by the mean square. A comparison with Fisher and Yates' Table 4 (7) shows that 3 values (marked x) are either under 5% or over 95%, in accordance with what might be expected among 23 tests. In other words, we obtain a good estimate of the square of the standard deviation by using the mean square 71.65, or 8.46 as a good estimate of the standard deviation which appears to be uniform for all the patients. Thus, there is a probability of only 5% that the maximum phenolphthalein value in repeated sham feeding tests will vary by more than $\pm 8.46 \times 2.069$=about 17 m. eq./1 from the true value. Correspondingly, the variation in the maximum Congo values will be ± 18 m.eq./1, in the chloride values ± 7.83 m.eq./1, and in the volume of secretion ± 7.1 cc.

Since the accuracy with which the concentration of pepsin may be determined according to the method of Mett is not quite satisfactory, the writer did not feel justified in expressing the reproducibility of the pepsin values in the same way as the other maximum values and therefore abstained from a corresponding calculation. Most of the pepsin values in Table 1, however, show fair agreement between the corresponding maximum figures.

As far as the volume of secretion is concerned, Flensborg (5) found that the variation increases day by day with increasing volume of secretion. Therefore, a direct estimation by means of the X^2 distribution is inapplicable. Instead, the estimation was carried out by means of the logarithms of the volumes of secretion. The justification of this procedure may be seen from the fact that such a correlation was not found between the individual mean values for log. volume of secretion and the corresponding mean squares. Accordingly, Flensborg found a probability of only 5% that the variations in the volume of secretion day by day exceeded the mean value by $^{+34.5\%}_{-25.4\%}$ The figures in the present study do not show a daily increase in the variation with increasing volume of secretion. Therefore, the value ± 7.1 was calculated. Basing the calculation on the log. volume of secretion, we find that the figures corresponding to Flensborg's will be the mean value $^{+57.4\%}_{-36.5\%}$ i.e. a somewhat more marked variation in the volume of secretion than seen in Flensborg's histamine test.

As apparent from the examples shown in Figs. 1-4, the configuration of the curves after sham feeding has been started is reproducible, the maximum values appearing to be particularly constant. Thus, they seem to be independent of the values found before sham feeding is started.

TABLE 1.

MAXIMUM VALUES FOUND BY SHAM FEEDING ON TWO DIFFERENT DAYS.

Maximum volume of secretion		Maximum Congo		Maximum Chloride		Maximum Pepsin		Maximum Phenolphthalein		Mean Phenolphthalein	Sum of Squares	X^2
I	II	I	II	I	II	I	II	I	II			
22	15	80	96	—	—	—	—	98	105	102	25	0,35
30	59	74	91	132	138	169	256	87	96	92	41	0,57
132	116	27	38	128	121	81	81	38	45	42	25	0,35
18	12	71	54	125	121	196	289	81	61	71	200	2,79
28	14	75	71	137	140	256	196	87	76	82	61	0,85
23	20	45	68	133	131	121	196	51	74	63	265	3,70
29	50	54	88	139	147	196	289	60	91	76	481	6,70 x
45	37	76	80	139	141	196	169	81	85	83	8	0,11
25	29	54	47	133	129	100	81	60	53	57	25	0,35
42	46	83	65	140	126	324	361	90	72	81	162	2,26
26	19	73	73	123	124	484	484	80	80	80	0	0,00 x
46	34	105	103	140	146	256	289	110	110	110	0	0,00 x
22	23	106	111	145	150	225	169	110	116	113	18	0,25
21	16	106	97	147	143	225	289	110	101	106	41	0,57
17	21	104	99	145	139	256	289	116	105	111	61	0,85
18	16	99	88	139	136	324	361	104	91	98	85	1,05
27	19	86	84	135	130	361	324	94	92	93	2	0,03
25	24	68	76	138	138	64	100	78	83	81	13	0,18
24	23	78	76	133	138	100	144	82	83	83	1	0,01
72	69	78	82	132	135	121	100	83	87	85	8	0,11
18	24	88	92	137	142	484	441	97	102	100	13	0,18
19	22	96	104	139	144	484	196	103	112	108	41	0,57
25	28	69	58	124	126	256	289	76	64	70	72	1,00

Degrees of freedom 23 Mean square: 71.65

REPRODUCIBILITY FOLLOWING OPERATION

To test the applicability of the sham feeding procedure following gastric surgery, 5 experiments were carried out on ulcer patients before and after gastro-entero-anastomosis. This operation does not remove gastric tissue which may influence the acidity of the gastric juice, although it may involve an increased admixture of extragastric secretions.

Table 2 gives the maximum values from these 5 experiments and Fig. 5 illustrates one of the experiments before and after operation. It will be seen that the volume of secretion is subject to some alteration following gastro-entero-anastomosis, since in some experiments part of the secretion seems to have passed into the intestine, whereas in others its volume has been changed by an increased admixture of bile. None the less, no major alterations have occurred in the

TABLE 2

MAXIMUM VALUES IN SHAM FEEDING TESTS BEFORE AND AFTER GASTRO-ENTERO-ANASTOMOSIS

Maximum volume of secretion		Maximum phenolphthalein		Maximum chloride		Maximum pepsin	
before	after	before	after	before	after	before	after
50	17	78	71	130	123	144	225
34	70	100	70	143	126	324	256
51	35	100	96	142	146	144	256
34	30	91	74	136	128	256	225
34	33	50	51	120	118	196	169

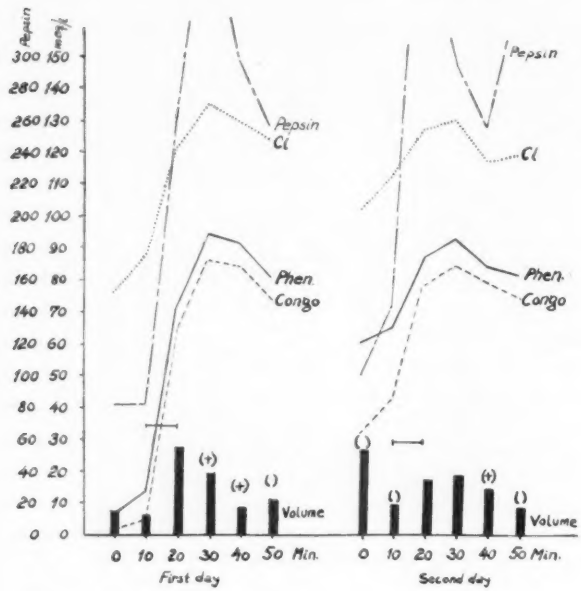

Fig. 2: Sham feeding test on the same subject at an interval of a few days.

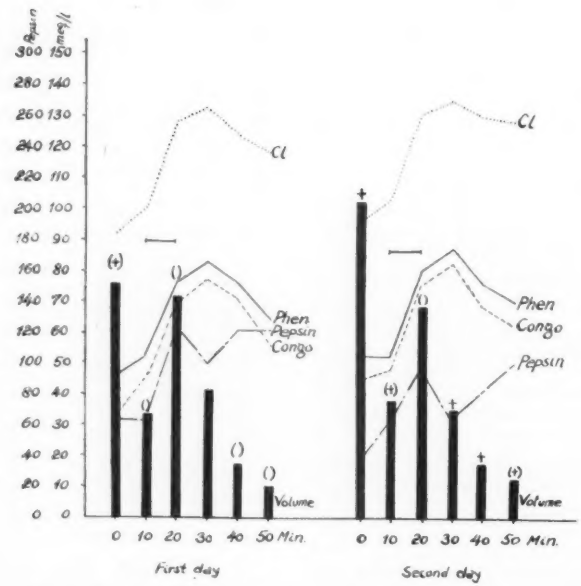

Fig. 3: Sham feeding test on the same subject at an interval of a few days.

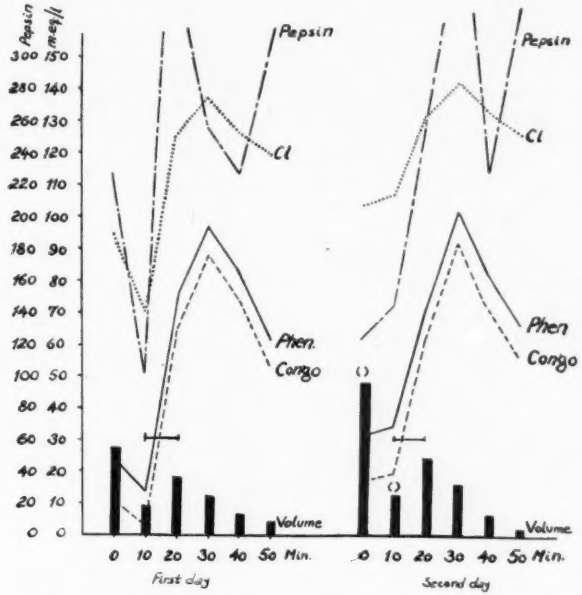

Fig. 4: Sham feeding test on the same subject at an interval of a few days.

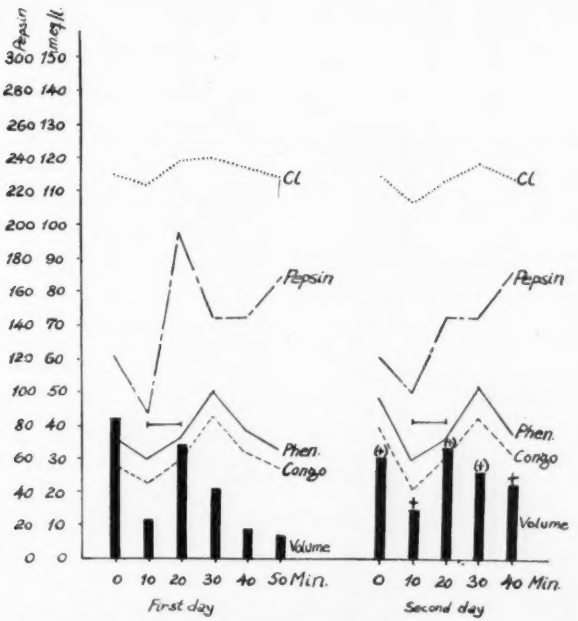

Fig. 5: Sham feeding test on an ulcer patient before and after gastro-entero-anastomosis.

acidity curves, the most marked feature of gastric secretion.

CONCLUSIONS AND SUMMARY

In an endeavor to test the reproducibility of the cephalic phase of gastric secretion, sham feeding tests were carried out on 23 subjects on two different days with an interval of 1 to 3 days.

It proved possible to reproduce the course of the curves representing the secretion as well as the maximum values. A calculation of the deviation in maximum values to be expected in repeated sham feeding tests showed a probability of only 5% that the maximum phenolphthalein value might vary by more than 17 m.eq./l from the true value.

Moreover, the sham feeding test appears to be easily reproducible following gastro-entero-anastomosis.

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THE BENZOIN AND SHELLAC TURBIDITY AS LIVER FUNCTION TESTS*

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ABERRATIONS or alterations of blood protein are generally recognized as the biochemical basis for the majority of the turbidimetric or flocculation liver function tests. The serum protein change is considered to be predominantly in the *globulin fraction*. Because of these observations, Fischer and Wiltner (1) felt it might be advantageous to apply the shellac and benzoin tests for spinal fluid globulin determination, to that of blood globulin.

Staub and Jezler (2) reported non-specific precipitation when water or sodium chloride solutions were used for dilution. Fischer and Wiltner confirmed these findings and observed that when suitable buffer solutions were employed reactions occurred which could make these tests useful in revealing liver damage. Their evaluations of these tests in patients with liver disease prompted the authors to conclude that further studies were warranted.

The purpose of our study was twofold: namely, to evaluate these substances as possible liver function tests and, secondly, to compare them with other flocculation and turbidity liver function tests as the cephalin-cholesterol, thymol turbidity and zinc turbidity.

MATERIAL

In order to carefully evaluate these tests a varied group of patients was studied. They included known normal hospital patients and allegedly normal individuals as represented by 100 tests performed on blood specimens obtained from donors at The Junior League Blood Center of Milwaukee. The pathological group consisted of patients with primary liver disease, obstructive jaundice, and systemic diseases which may produce liver dysfunction and/or alteration in blood proteins. A total of 355 tests were performed on this group; each patient had a shellac and a benzoin test performed.

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PROCEDURES

SHELLAC LIVER FUNCTION TEST:

Solutions Necessary: A stock solution of orange shellac was prepared by suspending 10 grams of shellac in 100 cc. of absolute alcohol. The solution was allowed to stand for 24 hours, with occasional shaking during this period, and then filtered.

Two solutions were necessary to prepare the phosphate buffer. Solution "A" was prepared in the following manner: 9.078 grams of potassium phosphate (KH_2PO_4) were dissolved in 1000 cc. of distilled water, Solution "B" was prepared as follows: 11.076 grams of sodium dibasic phosphate (Na_2HPO_4) were dissolved in 1000 cc. of distilled water. Seven (7) parts of solution "A" were mixed with three (3) parts of solution "B" in the preparation of the buffer solution.

Test Procedure: Serum from fasting patients was used for each test. Four test tubes were required. In the first tube 0.05 cc. of serum and 0.95 cc. of phosphate buffer solution were pipetted. To the remaining tubes 0.5 cc. of the buffer solution was added. The contents of the first tube were thoroughly mixed, and 0.5 cc. of this solution was transferred to the second tube. This procedure was repeated for the 3rd and 4th tubes, and the excess 0.5 cc. from the 4th tube was discarded.

With each group of tests a control was kept which consisted of 0.5 cc. of buffer solution to which 1 cc. of shellac solution was added.

One (1) cc. of a freshly prepared shellac solution was then added to each of the 4 tubes. This solution was made by adding 1 cc. of the stock shellac solution to 4 cc. of absolute alcohol, and the resulting product was poured rapidly into 20 cc. of distilled water. The tubes were thoroughly shaken and allowed to stand at room temperature for 18 to 24 hours. Positive reactions were read from 0 to 4+, depending upon the degree of turbidity, to complete precipitation with clear supernatant fluid. A 1+ turbidity was considered as a negative reaction.

BENZOIN LIVER FUNCTION TEST:

Solutions Necessary: A stock solution of Benzoin resin was prepared by suspending one gram of Sumatra benzoin resin in 10 cc. of absolute alcohol. The mixture was allowed to stand for 48 hours and then filtered. A barbital buffer solution was prepared by dissolving, with the aid of gentle heating, 0.55 gram of barbital and 0.31 gram of sodium barbital in 100 cc. of distilled water containing 0.2 gram of phenol.

Test Procedure: Serum from fasting patients was used. For each test, four test tubes were required. In the first tube, 0.05 cc. of serum and 0.95 cc. of barbital buffer solution were pipetted. To the remaining tubes 0.5 cc. of the buffer solution was added. The contents of the first tube were thoroughly mixed and 0.5 cc. of this solution was transferred to the second tube. This procedure was repeated for the 3rd and 4th tubes and the excess 0.5 cc. from the 4th tube was discarded.

With each group of tests a control was kept which consisted of 0.5 cc. of buffer solution in which 2 cc. of colloidal benzoin solution was added.

Two cc. of a freshly prepared colloidal Benzoin solution was then added to each of the 4 test tubes. This preparation was made daily by adding 0.3 cc. of the stock Benzoin resin, drop by drop, with constant shaking to 20 cc. of distilled water previously heated to 35°C. The tubes were thoroughly shaken and allowed to stand at room temperature for 18 to 24 hours. Positive reactions were read from 0 to 4+, depending upon the degree of turbidity with visible precipitation, to complete precipitation with clear supernatant fluid.

RESULTS

A. *Blood Center Controls*: Of the 100 cases studied (Table 1), sixty-seven revealed a normal shellac and benzoin. Thirty-three blood specimens produced an abnormal shellac and/or benzoin. Further analysis of the 33 abnormals revealed that 11 samples yielded abnormal reactions to both tests; 20 had an abnormal reaction to shellac and normal to benzoin; and 2 had an abnormal reaction to benzoin, but a normal reaction to shellac. In 10 instances of an abnormal shellac or benzoin reaction, a simultaneous zinc sulfate turbidity test was done with an abnormal finding in each instance.

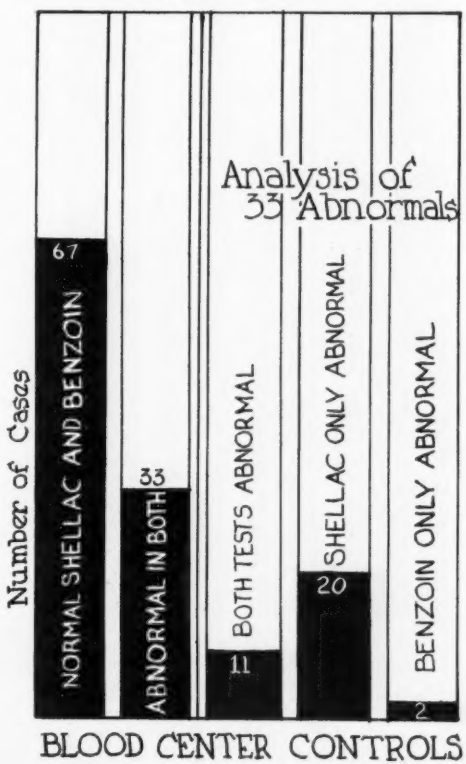
B. *Diseases of the Liver*:

1. Acute Hepatitis: In cases tested (Table II), the shellac turbidity was abnormal in 88% (15 of 17 tests), and the benzoin turbidity was abnormal 70.5% (12 of 17 tests). These findings compared with a positive reaction (2+ to 4+) in 50% of the cases, using the cephalin-cholesterol flocculation (5 out of 10), and 92% using the thymol turbidity (13 out of 14 tests).

2. Portal Cirrhosis: A shellac and benzoin turbidity test was performed 50 times. Eighty-six percent (43 out of 50) produced an abnormal shellac, while 76% (38 out of 50) resulted in an abnormal benzoin (see Table II). Comparative studies revealed 83% positive (25 out of 30) using the cephalin-cholesterol flocculation; 90% positive (32 out of 36) using the thymol turbidity test. The frequency of obtaining a positive reaction in the jaundiced cirrhotic was no greater than in the non-icteric patient.

3. Obstructive Jaundice: Unfortunately, we were able to perform our tests on a limited number of patients with this condition (Table III). However, we believe that it is significant that not a single case of the seven studied produced a positive test. In comparison with the other flocculation tests it was noted that identical results were secured using the zinc sulfate turbidity, whereas the results were less reliable using the cephalin-cholesterol flocculation or thy-

TABLE I



mol turbidity. Needless to say, a larger series would be of greater statistical import.

4. Unselected Cases: A total of 227 tests were done on patients with miscellaneous diagnoses (Table IV). 128 produced abnormal shellac and benzoin; 93 yielded an abnormal shellac, and 6 had an abnormal benzoin and normal shellac. None of these patients were believed to have primary liver disease. A great percentage could very well have had secondary liver disease and/or protein alteration.

ANALYSIS OF RESULTS:

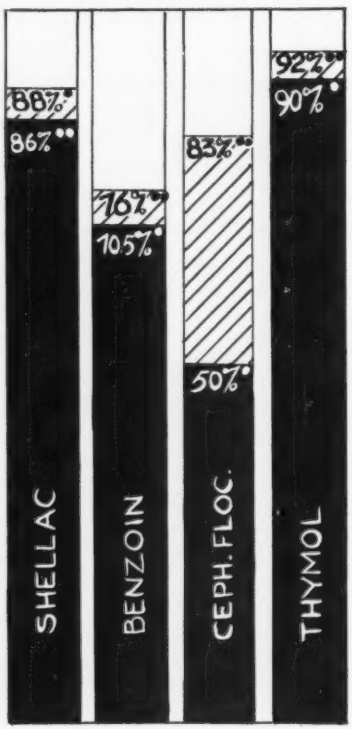
In the group of controls studied, one is readily impressed with the high incidence of positive tests. In comparison with the Blood Center studies, we feel that the percentage of positive results is too high, thereby indicating a high false positive group, possibly due to non-fasting blood samples, or probably due to a markedly sensitive testing. To determine what influence, if any, the non-fasting state had on the results of the test, eight patients who were having cholecystograms performed had a blood specimen drawn both before and four hours after eating the fatty meal usually given in performance of the Graham-Cole test. No difference was noted in the results of the fasting and non-fasting specimens.

In patients with liver disease the shellac turbidity compared favorably with that of the thymol turbidity and was considerably more reliable than the cephalin-cholesterol flocculation test in acute hepatitis. The benzoin test was distinctly less frequently positive than either the thymol turbidity or the shellac turbidity and more frequently positive than the cephalin-cholesterol in the cases of acute hepatitis.

In individual instances of the latter group, negative results were obtained in rather severe or advanced cases of acute hepatitis.

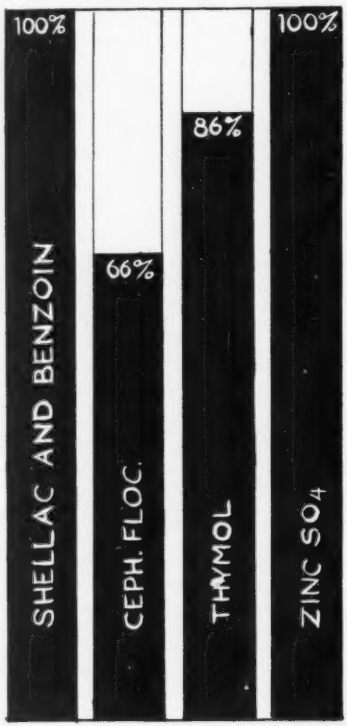
Our findings in obstructive jaundice were very favorable and equaled that of the zinc sulfate turbidity, but the series was too small to be positively significant.

TABLE II



*ACUTE HEPATITIS
(Positive results in 17 cases)
**PORTAL CIRRHOSIS
(Positive results in 50 tests)

TABLE III



OBSTRUCTIVE JAUNDICE
Negative tests in 7 cases...

Further studies of these tests in this group would be warranted.

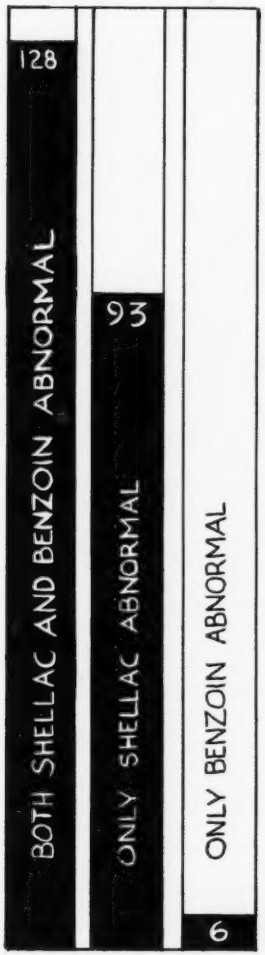
Analysis of the miscellaneous group revealed again a rather high percentage of unexpected positive findings particularly with the shellac determination. Reasons for this are not definitely clear.

DISCUSSION

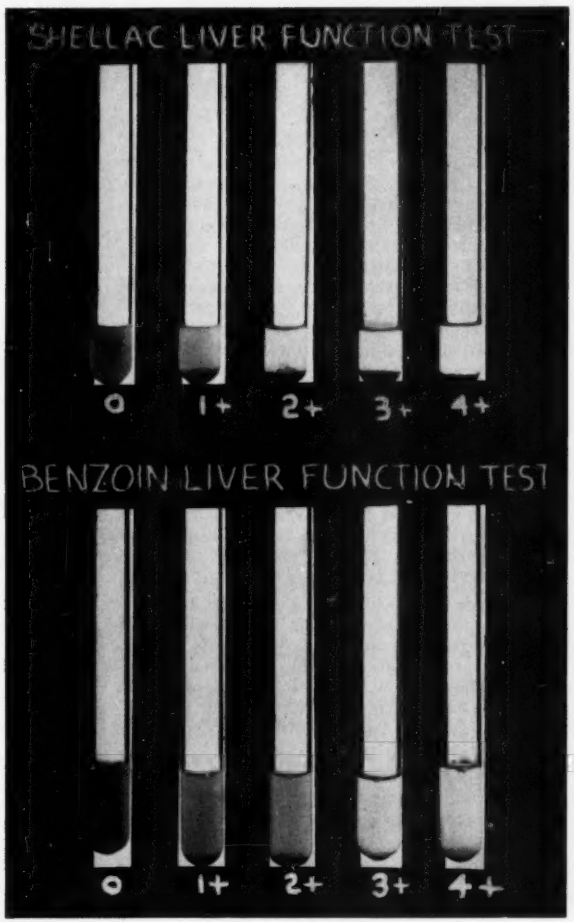
The tests as originally described by Fischer and Wiltner seemed impractical to us and warranted the following two major modifications: (1) As a result of a comparative study, orange shellac was used in preference to the bleached shellac in preparation of the stock solution in order to obtain a more sharply defined end point, and (2) the time interval for reading the shellac turbidity test was changed from 12 hours to 18 to 24 hours to make the test adaptable to routine laboratory use.

The principal objection to the tests was a long delayed interval before reading the results. This feature

TABLE IV.



MISCELLANEOUS CASES



made them inferior to the thymol and zinc sulfate turbidity tests.

The shellac test compared favorably with the thymol turbidity in cases of acute hepatitis and portal cirrhosis, and proved superior to the cephalin-cholesterol flocculation test. Further modification of this test may make it practical for utilization as a confirmatory test in cases of liver infection and/or dysfunction.

SUMMARY

1. The Shellac and Benzoin Turbidity Liver Function Tests with modifications are described.
2. The results in controls, acute hepatitis, portal cirrhosis, obstructive jaundice and miscellaneous conditions are given.
3. A comparison is made with the thymol turbidity and the cephalin-cholesterol flocculation test.
4. A larger series of cases of obstructive jaundice should be studied to further evaluate the tests.

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ON THE RELATION OF FUNCTIONAL HYPERINSULINISM TO VISCEROPTOSIS

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SINCE MY ARTICLE "On the Relation of Functional Hyperinsulinism to Visceroptosis" appeared in the October, 1951 issue of *"The American Journal of Digestive Diseases,"* I have had thirty-two patients with gastroptosis undergo the four-hour glucose tolerance test. My first article, with twenty-one cases, was mainly concerned with demonstrating that gastroptosis causes hyperfunction of the pancreas. In this paper with the additional thirty-two new cases, making a total of fifty-three cases, I shall attempt to interpret the varying forms of hyperinsulinism in these cases in accordance with recent investigations on the very complex problem of carbohydrate metabolism in body and muscle. We shall also see that hyperinsulinism cannot be measured by the degree of hypoglycemia alone as the hypoglycemic effect may be counter-balanced by the opposing but not antagonistic effect of adrenalin hormone. Hyperinsulinism may even co-exist with high fasting blood sugar and hyperglycemia in some phases of the glucose tolerance test and even with glycosuria with low blood sugar threshold.

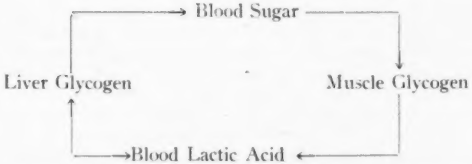
The final hypoglycemic effect depends on the individual functional capacity of the pancreas and other hormones especially the adrenalin and second on the form of the gastroptosis which gives rise to the disturbance. A brief summary of the anatomic variations and the radioscopic classification of gastroptosis as presented in the French textbooks will help to clarify the different types and their possible reaction on the pancreas.

According to Cunningham's *Text-Book of Anatomy* the position of the digestive organs in healthy living persons is much more variable than might be supposed. Observations made by R. O. Moody and his co-workers on students in California and in London confirm the view that in the living, the form and position of the stomach vary in different people and even in the same person with changes in posture. The individual variations which may occur are of wide range.

The pylorus may lay as high as the upper half of the 12th thoracic vertebra or as low as the upper half of the 5th lumbar vertebra and below the "inter-iliac" line or plane of the iliac crests. It may lie in the median plane or to the right or to the left of it. Similarly the greater curvature of the stomach may lie as high as the 12th thoracic or as low as the first sacral vertebra. Associated with these different positions of the pylorus there is a difference in the position of the first part of the duodenum and in its relation to the head of the pancreas.

According to French clinical text-books the radioscopic characters of gastroptosis are classified as follows: I. Incomplete ptosis. Habitus Enteropticus. J-shaped stomach without atony or dilatation. On the lesser curvature the notch is hook-shaped. II. Moderately atonic and ptotic stomach. Diaphragm is lower on the left side, pyloroptosis, acute angular notch of the lesser curvature and the greater curvature descends largely below the inter-iliac plane. III. Bilocular stomach, very atonic and dilated with ptosis of the elongated duodenum. The whole stomach lies on the left side of the vertebra on the pelvic floor. IV. Complete ptosis with loosened connections of the stomach with the diaphragm. The diaphragm has the form of a roof over the stomach. The preceding anatomic and radioscopic outline of the different types of gastroptosis makes it readily conceivable why gastroptosis does not affect different individuals with gastroptosis in the same manner.

In order to better comprehend and interpret the neurological symptoms of gastroptosis with hyperinsulinism, a brief summary of the physiological and biochemical principles governing the carbohydrate metabolism in human body is indicated. The physiological influences govern the fate of glycogenolysis in the muscles; one is adrenalin from the adrenal medulla; the other is muscular exercise. Adrenalin in concentration as low as one part to 100,000,000 parts of Ringer's solution increases glycogenolysis in frog muscle. Insulin, while it causes formation of muscle glycogen from glucose, does not inhibit the action of adrenalin during muscular activity. Glycogen yields lactic acid which pours into the blood and is taken up by the liver. In the liver lactic acid is converted to glycogen and serves as a source of blood sugar according to the *Coricycle*.



Lactic acid plays an important part in the production of fuel for utilization in the body for the production of mechanical work. When the muscle contracts in the absence of oxygen glycogen disappears continuously and the corresponding amount of lactic acid appears. After a time the muscle reaches a condition of rigor and will not respond to stimuli. In the presence of oxygen lactic acid disappears and carbon dioxide is produced. The process of the breakdown of glycogen to lactic acid yields about 180 small calories per gram of lactic acid formed. The formation of lactic acid from glycogen liberates the energy for resynthesis of phosphocreatine. Phosphocreatine occupies a vital part in the conversion of energy derived from glucose into work in form of muscular contraction.

Muscular contraction may proceed as follows:

I. A T P (Adenosine triphosphate) supplies the energy for the actual contraction by forming adenylic acid and phosphorylated myosin.
A T P plus Myosin ——→ Adenylic acid plus phosphorylated myosin.

II. Phosphocreatine which serves as a reservoir to replenish A T P and then breaks down to supply the energy and phosphate for the resynthesis of A T P.
Phosphocreatine plus adenylic acid ——→ Creatine plus A T P.

III. Phosphocreatine is then resynthesized by the normal process of phosphorylation, lactic acid also being formed.
Creatine plus 2-phosphopyruvic acid ——→ Phosphocreatine plus lactic acid.

IV. About one-fifth of the lactic acid formed above is then oxidized to furnish energy for the resynthesis of glycogen from the rest of the lactic acid.

The process whereby the "fuel" is oxidized to yield energy to be utilized for work is called carbohydrate metabolism. The breakdown of a molecule of glucose or fructose to yield carbon dioxide and water may be simply written thus:

$C_6H_{12}O_6$ plus 6 O_2 ——→ 6 CO_2 plus H_2O plus Energy

Starch and glycogen are polymers of glucose. These substances are storage forms of fuel in which acetal type bonds (about 3 kilocalories) already exist and then they are acted upon by the enzyme phosphorylase in the presence of adenylic acid (adenosine monophosphate) and inorganic phosphate they are converted to glucose-l-phosphate often called the Coriester without any expenditure of energy.

The few physiological and biochemical facts mentioned will enable us to understand better the symptoms of visceroptosis and hyperinsulinism. Let us examine first the graph forms of the four-hour glucose tolerance tests. We see that some of the graphs have a flat shape which means that peaks of the blood sugar levels rise only a few points over the fasting blood sugar and the following drop is also only a few points below the fasting level. The fact that the blood sugar did not mount to an appreciable extent during the test proves that there must be a considerable supply of insulin to be able to convert without delay the glucose into glycogen and storing it away in the liver and the muscles. As soon as the surplus sugar is eliminated and hypoglycemia results, the hypoglycemia itself acts as a stimulus to glycogenolysis directly or indirectly in that it causes adrenaline secretion.

I noticed that patients with flat type graphs suffer especially from torturing apprehensions of all kinds. They are afraid to cross the street, to go out of the house or even to go to bed. Usually these patients were treated by the profession as psychosomatics, conversion hysteria, etc. The fact that the fears disappeared when the hypoglycemia symptoms were corrected makes it plausible that the abnormal fears may be caused from adrenalin production in the blood which in turn is caused by hyperinsulinism.

CHART I

9 CASES WITH GLYCOSURIA

I—No. 14 L		Time of Glycosuria 1.0g sugar in 1 hr. per 100cc Urine	Time of Glycosuria Blood sugar at 1 hr.	Time of Highest blood sugar ½ hr.
			142	145
Case 19 Chart I Joseph Haffner	3/13/51			
without support		Less 0.5gr. ½ hr.	118	148
with support	3/20/51	None		138
II—No. 1. without support	2/28/51	At ½ hr. less than 0.05 at 1 hr. At 2 hr.: 0.75 sugar	131 135 120	135 1 hr.
with support	4/10/51	At 2 hrs.: less than 0.5 gr.	126	130 1 hr.
No. 4. A.W.	5/5/51	Less than 0.5 gr. ½ hr.	185	193 1 hr.
No. 7. Less than 0.5 gr. R.C.	6/4/51	Less than 0.5 gr. 3rd hr.	65	½ hr. 135
No. 10. M.L.	6/26/51	Less than 0.5 gr. 0.5 in 1 hr. Less than 0.5 gr. 2 hrs.	178 140 122	178 in. ½ hr.
II No. 16 E.P.	8/14/51	Less than 0.5 gr. in 1 hr. and 2 hr.	121 90	H. ½ hr. 140
No. 20 R.T.	8/27/51	1% in 1 hr. with support	142	H. ½ hr. 151
	11/20/50	None		123
No. 26	9/26/51	1 hr. 150	150	¼ hr. 162

A brief remark about the column in the chart which contains the negative or positive numbers. They represent the net results of the end of the four-hour glucose test when the blood sugar values over the fasting levels designated as positive are added to the blood sugar levels below the fasting level designated as negative. Contrary to the opinion found in the medical textbooks that patients with hyperinsulinism are often obese, my patients are often 30 or 40% underweight in spite of their eating larger amounts of food than usual. As a matter of fact, many underweight patients consulted me primarily because of their inability to keep their weight normal.

This paradoxical phenomenon can readily be understood when we recall what happens in hyperinsulinism to the blood sugar according to the Coricycle. The glucose of the blood, including the glucose which was present at fasting, is transformed to glycogen through the action of hyperinsulinism and stored away mostly in the muscle. Through physiological influences of muscular exercise or adrenaline, glycogenolysis is brought about. After ten biochemical reactions termed phosphorylation pyruvic acid is formed which gives rise to lactic acid. Lactic acid is used partly as pH control in the blood, secreted by the sweat glands and when the content rises in the blood it may be excreted by the urine. The greater part is transported to the liver where it is resynthesized to glycogen and then to glucose to maintain the blood sugar level. Now in hyperinsulinism the glucose that has just gone through the Cori-Coricycle may again be stored in the muscle as glycogen and repeat the cycle again from lactic acid to glucose and again to lactic acid. We can readily see how uneconomical this abnormal carbohydrate metabolism is. This abnormal physiochemical condition in the muscle may be the cause of the myalgias about which the patients with gastroptosis so often complain. According to Damshek (No. 3, Bibliography list) hypoglycemia is accompanied by accumulation of lactic acid and lack of oxygen in the brain. This explains the diversity of neurological symptoms and complaints which are encountered in these conditions.

NEUROLOGICAL SYMPTOMS

The brain and its physiological centers and the endocrine glands must perforce be affected both by the hypoglycemia and by the accompanying lack of oxygen. The secretion of other pancreatic hormones as vagotonine, padutin, etc., may be affected in the same way as the islets of Langerhans and be stimulated to hypersecretion. This may explain the frequent vagotonic symptoms encountered in gastroptosis.

In examining these patients we are often impressed with the multifarious neurological symptoms about which they complain. These range from simple fatigue and "indescribable" feelings in the head to severe headaches, loss of consciousness and convulsions. Fears

CHART II

4-HOUR GLUCOSE TOLERANCE TEST

Date	Name of Patient	Age	MG/100cc Fasting	MG/100cc ½ Hr.	MG/100cc 1 Hr.	MG/100cc 2 Hrs.	MG/100cc 3 Hrs.	MG/100cc 4 Hrs.		Remarks
No. 1.										
4-10-51	Martin Sunshine	62	104	115	130	126	46	73	—30	Urine: Less than 0.5gr. sugar per 100cc. in 2 hr. test. Wore support not properly adjusted.
2-28-51			111	131	135	120	85	64	—20	Without support
No. 2.										
5-1-51	Edward Kushner	30	112	122	128	114	83	85	—28	
No. 3.										
5-9-51	Dana Parsons	29	114	180	130	104	87	102	+33	
No. 4.										Urine: less than 0.5 gr. per 100cc.
5-15-51	Mrs. Anna Wilk	36	100	185	193	160	116	72	+226	Urine in ½ hr. and 1 hr. test. Height 4'10".
No. 5.										
5-15-51	Mr. H. Whitman	65	91	155	134	119	99	64	+116	Was wearing support for 2 yrs. Not during test.
No. 6.										
6-1-51	Mrs. Ruth Shaw	32	86	145	75	83	89	56	+18	
No. 7.										Urine: less than
6-4-51	Mr. Ross Camerotti	30	109	135	130	124	65	93	+1	0.5 gr. of sugar in 100cc. urine.
No. 8.										
6-14-51	Elaine Schlachter	14	116	130	100	89	83	72	—106	
No. 9.										
6-19-51	Mrs. Pearl Amber	39	99	112	101	75	97	83	—27	
No. 10.										Urine: ½ hr. less than 0.5cc. sugar.

6-26-51	Mrs. Mildred Licker	45	105	178	140	122	139	88	+142	1 hr: 0.5 gr. per 100cc. urine 2 hr: less than 0.5 gr. per 100c. urine.
No. 11.										
7-2-51	Mrs. Esther Wolf	46	100	142	136	85	78	83	+24	
No. 12.										
7-6-51	Helen Semowsky		99	140	149	79	69	80	+22	
No. 13.										
7-12-51	Mrs. Lucille Schoem	36	99	142	108	87	105	72	+19	
No. 14.										Was wearing sup-
7-20-51	Mrs. Frank Schuesler	46	99	144	138	119	62	93	+61	port for 4 yrs. but not during t. t.
No. 15.										
8-7-51	Mrs. Threscher	48	116	125	125	109	107	106	—8	
No. 16.										Less than 0.5 sugar
8-14-51	Elizabeth Prayner	54	84	140	121	90	83	77	+91	in urine in 1 hr. test.
No. 17.										
8-15-51	Joseph Pecek	15	101	140	104	110	64	100	+13	
No. 18.										
8-19-51	Mr. E. L. Foust	65	104	146	173	84	72	69	+24	
No. 19.										
8-22-51	Helen Adams	19	80	164	142	102	78	60	+146	
No. 20.										1% sugar in urine
8-27-51	Ralph Thiesse	23	86.1	151	142	73	53	78	+66.5	0.3 gr. at 1 hr. Was wearing abdominal support.
11-29-50			96.1	123	108	87.3	79	80.6	—2.6	Without support. Is 6'4'' tall.
No. 21.										Worker with strong
8-30-51	H. Gentry	28	80	137	128	96	72	81	+114	abdominal muscles Incomplete ptosis.
No. 22										
9-11-51	Miss O'Laughlin	43	93	103	114	94	72	90	+8	
No. 23.										Has good stomach
9-12-51	Miss Stern	34	89	114	90	107	90	90	+46	tonus type I. Incomplete ptosis.
No. 24.										
9-19-51	Dan Siwick	23	97	136	124	86	79	69	+9	Ex Marine.
No. 25.										Ex Marine.
9-25-51	George Martin	28	134	192	126	136	55	76	—85	6'4''.
No. 26.										0.2% sugar in urine
9-26-51	Mrs. Ida Wilson	46	103	162	150	72	76	47	—8	Total 0.1 gr. after 1 hr.
No. 27.										
10-3-51	Mrs. Dragin	48	104	113	94	84	105	78	—46	
No. 28.										
10-10-51	Miss Mary Schiller	34	90	127	99	82	78	67	+3	
No. 29.										
12-5-51	Mrs. Higgins (mother)	42	93	137	102	75	78	61	—12	
No. 30.										
12-5-51	Beatrice Higgins (daughter)	13	97	114	105	78	78	71	—39	
No. 31.										Fears and appre-
12-21-51	Mrs. B. Whaye	48	97	102	83	80	72	67	—81	hensions all her life not focused to any object.
No. 32.										Was wearing ab-
12-28-51	Mrs. M. Elsoffer	24	102	143	97	95	87	75	—13	dominal support for 4 yrs. but not during gt. t

in any form at anytime of day or night and at any place are very often encountered in gastroptosic patients. The fears are characteristically detached and not fixed to any object. These sensations of fear are presumably physicogenic and may be caused by the production of adrenalin in the blood to balance the hypoglycemia. Next in frequency of the neurological symptoms is a peculiar kind of headache combined with a feeling of panic which may persist for several days; this feeling of panic is one which patients dread most. These headaches are probably also physicogenic, caused by hypoglycemia and lack of oxygen in the brain.

Loss of consciousness is less frequently experienced; only ten percent of my patients had it. One patient (aged 60), had at the age of twenty a sudden attack which never recurred. Another patient (aged 35), had several attacks at the age of twelve, but has had none since that time. A third patient had frequent attacks before measures were taken to correct the ptosis. A few more patients had fainting spells occasionally but these spells have not recurred since gastroptosis has been treated with support and diet. Only two patients, both in their teens, had convulsions. From the number of patients studied, an important fact stands out—the patients with more severe hypoglycemia did not have loss of consciousness or convulsions. The patients with pronounced hypoglycemic phases complained of spells of extreme weakness and drowsiness. Some nar-

rowly escaped serious accidents after being overcome with drowsiness while driving their car.

One symptom which to my knowledge is not mentioned in connection with any other disease and which seems to be almost pathognomonic for gastroptosis with hyperinsulinism, merits special consideration. These patients often get dizzy and faint when ascending or descending the stairways. The school children especially notice that they have to hold on to their friends or the rails each time they go up or down stairs. One mother (42) with normal blood pressure fell downstairs three times getting dizzy while her daughter (13) had to hold on to the rails each time she went up or down stairs. Both have similar type of gastroptosis and graphs of the glucose tolerance test. We can readily imagine that the same thing may happen when a flyer changes altitude suddenly.

The organism can regulate the blood sugar levels to safeguard against extreme hypoglycemia by speeding up glycogenolysis in muscle and liver through the adrenal secretion. A very effective way to prevent too low blood sugar is to keep the fasting blood sugar relatively high. The pattern of this regulatory mechanism is represented in the fasting blood sugar level and the glucose tolerance test curve.

A characteristic example is case No. 25, G. M. This patient had a hypoglycemic drop in the third hour of 79. If this patient would have had an average fasting blood sugar of about 90 or 80, the blood would have been suddenly deprived of all of its sugar and could have been fatal. By the rising of this individual's fasting blood sugar to 134, the blood sugar in the third hour, after a drop of 79 points, was 55. This regulatory mechanism may be the cause for other cases in the chart with comparatively higher fasting blood sugar levels.

GASTROPTOSIS WITH GLYCOSURIA

Gastroptosis with hyperinsulinism is a new syndrome and the whole symptomatology is not charted yet. I consider it especially interesting to study in a separate group those cases which exhibited glycosuria in some phases of the test. As the chart indicates, there were nine cases showing traces of glycosuria during the test. A few of these patients with higher blood sugar levels, previous to urine tests, might be considered as potential or mild diabetics. This combination of mild diabetes with gastroptosis and hyperinsulinism is especially noteworthy. There are a few cases, as can be seen from the chart, which showed glycosuria at a very low threshold.

It is known that normal individuals may secrete 142 milligrams of sugar in 24 hours. The glycosuria at low blood sugar threshold in so great a percentage of the cases may be due to disturbed phosphorylation in the renal tubuli which control the blood sugar threshold by reabsorption of the sugar and phosphorylations.

TREATMENT

A patient suffering from gastroptosis with hyperinsulinism with symptoms usually dating back to early teen age, and experiences of numerous therapeutic failures, assumes a diffident attitude and believes that partial temporary relief through sedatives is his only remedy.

I start, therefore, by reassuring the patient that his symptoms are not due to organic causes but to a physical inadequacy which in turn provokes functional hormonal disturbances. The confidence and understanding cooperation of the patient is essential for the successful execution of the regimen. I then proceed in impressing upon him the importance of the proper fitting of the support, as otherwise it may even aggravate his condition instead of improving it. Physical exercises to strengthen the abdominal muscles and the tonus of the viscera is then urged. These physiotherapeutic measures cannot perforce totally correct all forms of ptosis, especially in tall people with atonic stomach and pyloroptosis. Therefore, dietary measures are also necessary.

DIETARY MEASURES

In the medical literature, a diet for hyperinsulinism is recommended which should contain low carbohydrate (70-80 gm.), high fat (100-150 gm.), and moderate protein (70-90).

In my cases of gastroptosis with hyperinsulinism, I found it not necessary to drastically restrict the normal eating habits of the patient. He is only advised to abstain from sweets and pastries, ice cream sodas, etc. In especially underweight patients, a glass of milk and cream or even a meat or cheese sandwich between meals and before bedtime, is very satisfactory.

SUMMARY

A new syndrome gastroptosis with hyperinsulinism is formulated. The twenty-one consecutive cases published previously and the thirty-two cases in this paper comprise all forms of gastroptosis.

The final forms of the graphs of the four-hour glucose tolerance tests depend:

I. On the form and degree of the ptosis.

II. On the individual physiological capacity and behavior of the pancreas.

III. On the balancing influences of other hormones, especially the adrenals and the nervous system.

The definition of hyperinsulinism is enlarged to include some cases where the elevation of the blood sugar level during the glucose tolerance test is noticeably suppressed with or without following hypoglycemia of appreciable nature. This was found confirmed clinically from the symptomatic improvement the patient derived from the regimen for this syndrome while no other therapeutic measures were of any benefit. Circumstances did not allow me to have the glucose tolerance test extended to six hours or even in some hospitalized patients to twenty-four hours. This would have shed more light on this new clinical field.

It would also be of great value to study this syndrome in flyers, truck drivers, locomotive engineers, etc., under regular diet and working conditions.

I wish to stress that gastroptosis and hyperinsulinism is not a rare occurrence. This can readily be seen from the fact that in the last three months I had

thirty-two cases. I leave it to the individual imagination to calculate how many cases there might be in this country.

CONCLUSION

These more than fifty (50) cases of gastroptosis with hyperinsulinism in various forms justify the assumption that gastroptosis with hyperinsulinism represents a distinct clinical entity and syndrome. Gastroptosis can readily be diagnosed in the physicians's office even without X-ray and the possibility of hyperinsulinism must then be considered and confirmed by a four-hour glucose tolerance test.

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4. Fraitè De Pathologie Medicale, Emile Sergent, L. Ribadeaux Dumon, et al.

ABSTRACTS ON NUTRITION

ROSENMAN, R. H., FRIEDMAN, M. AND BYERS, S. O.: *Observations concerning the metabolism of cholesterol in hypo- and hyperthyroid rats.* Circulation, V. 4, April 1952, 589-593.

The administration of thiouracil induced a significant rise and thyroid substance a significant decrease of the plasma cholesterol concentration in rats. The concentration and daily output of cholesterol was considerably reduced in bile of hypothyroid rats and markedly increased in the bile of hyperthyroid rats. The hyperthyroid rat was found to accumulate cholesterol in his plasma after biliary obstruction much more rapidly than the euthyroid or hypothyroid rat. This accumulation was found to be due to a far more rapid rate of manufacture or discharge of cholesterol by the liver of the hyperthyroid rat. The converse was found in the hypothyroid rat.

GOFMAN, J. W. AND JONES, H. B.: *Obesity, fat metabolism and cardiovascular disease.* Circulation, V, 4, April 1952, 514-517.

Of the two classes of serum lipoproteins which are strongly associated with atherosclerosis, the Sf 35-100 class is moderately related to obesity, whereas the Sf 12-20 class shows a much lesser, yet significant, relationship to obesity. The positive correlation of these classes of lipoproteins with obesity (and with atherosclerosis) provides possibly the largest part of the basis of the relationship of obesity with atheromatous vascular disease. This subtle relationship, however, is almost entirely obscured by the total serum cholesterol measurement. The control of obesity, as a prophylactic and therapeutic measure in arteriosclerosis, already a part of clinical practice, derives support from the finding of the association of certain lipoprotein classes with obesity.

JOHN, H. J.: *Further observations on the use of liberalized diets in the treatment of diabetes.* Ann. Int. Med., 35, 6, Dec. 1951, 1318-1328.

John has largely given up weighing diets. Patients are placed on diets averaging from 2,000 to 2,500 calories per day, which means, in fact, normal diets. Sweets and soft drinks are eliminated. Occasional ice-cream is permitted for its good psychological effect. However, John is very careful to see that the blood sugar just *before* meals is normal and uses enough PZI to accomplish this. Normal persons have elevated blood sugar about 25 percent of the time (during the absorption periods) and diabetics may be permitted the same phenomenon since it is physiological. Blood sugar estimations are more important than urinalyses for sugar. No one should attempt to treat glycosuria, for frequently there is sugar in the urine in the presence of a normal blood sugar. In beginning treatment he takes blood sugar readings before each meal to see whether or not the PZI has cleared the blood of abnormal sugar and, on this basis, he calculates the amount of insulin needed.

MUKHERJEE, C.: *Studies on carbohydrate metabolism in toxemia of pregnancy. 2. Blood sugar in toxemia of pregnancy with particular reference to eclampsia.* J. Indian Med. Assn., XXI, 5, Feb. 1952, 183-186.

Estimation of blood sugar was done in a series of 78 cases of normal pregnancy and 100 cases of pre-eclampsia. No significant change in the concentration of blood sugar was observed in the latter condition. A critical study of the results of blood sugar estimations done in various phases of eclampsia is presented. The results show that eclamptic convulsions are not in any way related to fluctuations in the blood sugar level.

PATEL, J. C.: *Necessity of B-complex factors in the treatment of anemias.* J. Indian Med. Assn., XXI, 5, Feb. 1952. 191-194.

In the various anemias seen in India, Patel has found it best to rely upon liver extract, folic acid or vitamin B_{12} without any additional substances. In iron deficiency, he uses only iron. He does not believe that the various factors in B-Complex, singly, have any effect on human anemia. Used in combination, in anemias, they may upset the enzyme equilibrium of the body. Liver extract, crude or refined, still remains a potent method of avoiding this accident while, at the same time, improving the anemia.

ALLAN, A. B.: *Treatment of pregnancy anemias with folic acid compound.* J. Indian Med. Assn., XXI, 5, Feb. 1952, 206-210.

The "Folic Acid Compound" used was prepared from partially digested yeast, mixed with liver concen-

trate so that each c.c. contains 0.5 mg. folic acid, 0.6 mg. of its conjugate and an extract from 15 gms. of liver. The usual daily dose was 2 c.c. Severe macrocytic anemias were selected. Most cases were complicated by intestinal and "blood" infections. The economic status of all the patients was very low, the diet having been extremely deficient in protein. The clinical response was good. The diarrhea was controlled and the edema subsided after 10 days of treatment. In most cases the blood count doubled in the first 10 days, however the F.A.C. seemed to have no further effect after the R.B.C. level reached 2.5 to 3 million cells per c.mm. The administration of iron then produced further improvement. Folic acid alone did not remove the macrocytosis. Liver and/or yeast contained essential factors needed. Allan feels that in India, at least, and among the poor, pregnancy anemia is a multiple deficiency disease.

MIURA, T., NAITO, K. AND ISHIZUKA, T.: *The excretion of vitamin C in the urine during and after the heating of the human body.* Med. J. Osaka Univ., 3, 4, June 1951, 55-60.

Two human subjects were kept for 3 hours in a high thermal environment, and the vitamin C (reduced form) excreted in the urine was measured. The excretion of vitamin C was diminished under these conditions, but on return to a normal temperature, the excretion was increased, after a time lag. This increase in excretion kept up for 6 hours. At present the role of ascorbic acid in the adaptation to high temperatures is obscure, but the present experiment suggests possible benefit of over-dosage with vitamin C under these conditions.

THOMSON, J. AND KEAY, K. R.: *A case of acute beriberi complicating polyserositis in Britain.* Brit. Med. J., Feb. 9, 1952, 295-298.

The authors report the case of the 12-year-old school girl who developed polyserositis (later shown to be due to tuberculosis) with pericardial and pleural effusions and hepatic enlargement, which responded to rest and streptomycin. However, she also developed beriberi because the use of thiamine caused rapid regression of her edema. (Later, because of constrictive pericarditis, a successful pericardectomy was done). The toxic process causing the polyserositis probably also produced the beriberi. No similar case has been discovered in the British literature.

OKAMOTO, K.: *Production of experimental diabetes mellitus and zinc reaction of Islets of Langerhans.* Hyogo J. Med. Sci., 1, 1, May 1951, 77-89.

Okamoto, in 1942, developed a new histochemical test for zinc in tissues, the Islets of Langerhans containing an appreciable amount of this metal, a fact which he believes is closely related to the production or progress of diabetes. Alloxan, oxine and dithizone are diabetogenic agents, each having an affinity for zinc. His hypothesis was that the degree of diabetes produced by these agents depended on the amount of zinc in the islet cells. Those diabetogenic agents cause severest diabetes in the rabbit and dog, and least severe diabetes in the cat and guinea pig, a fact which is parallel with the amount of zinc in the islets. Increased zinc was found in the islets after the use of anterior pituitary hormone, thyroid and adrenal cortical hormone, the production of diabetes being accelerated with the increased zinc content. Colored plates show rabbit and human pancreas stained for zinc by the diphenylthiocarbazide method.

ERICKSON, D. W. AND KING, R. L.: *Fluid administration: a simple method designed for patients confined strictly to bed.* Bull. Mason Clin., 5, 4, Dec. 1951, 112-113.

Discarded intravenous infusion flasks and plastic tubing is used, with a glass or plastic drinking tube on the end of the tubing and a pinch-cock, so that the patient is able to help himself to a drink at any time he wishes. By such oral, self administration of fluid, economy in nurses' time is gained and oxygen tents need not be opened.

YOUNG, F. B.: *The experimental approach to the problem of diabetes mellitus.* Brit. Med. J., Nov. 17, 1951, 1167-1173.

Young thinks that the growth hormone of the pituitary (not to be confused with ACTH) may be of importance in the etiology of a serious proportion of cases of human diabetes mellitus. Evidence at hand permits us to associate growth, including fetal growth, and milk production, as phenomena whose development is stimulated by growth hormone. Where growth hormone is injected into adult cats and dogs incapable of growth or milk production, diabetes results. In the human being, a slightly excessive production by the pituitary gland of growth hormone, insufficient to cause overt signs of acromegaly but lasting a long time, might lead to a restraint on carbohydrate and protein catabolism and oxidation, combined with increased appetite, and leading to diabetes. This line of reasoning is supported, to some extent, by the over-sized babies of women who later develop diabetes.

The diabetogenic action of growth hormone is regularly demonstrated only in certain adult carnivorous animals (cat, dog, ferret). In the *young* of these species, however, growth rather than diabetogenesis occurs in response to injection of growth hormones. In other words, excessive production of growth hormone by the pituitary is likely to produce diabetes only when growth is impossible (adults) or when lactation is not in progress (non-pregnant). The theory would seem to require a "frustration" of the usual effects of growth hormone before the latter could cause an arrest of oxidation of carbohydrate.

EDITORIALS

OVERWORKING THE PSYCHOSOMATIC CONCEPT

All good physicians from ancient times, including Hippocrates and Plato, were at all times keenly aware of the influence of ideas and emotions on illness and its treatment. The formalization of this awareness has been attempted in the past twenty years, especially, by various individuals and groups, who tried to apply scientific methods of measurement to the possible relationships existing between mind and body. It is a daily experience, to find a symptom-producing spastic colon in a person who is nervous or under stress, and to note disappearance of the symptoms (though not always the colonic spasticity) once the stress has abated. In some persons, colonic pain is present for years without any roentgenographic evidence of spasticity. The emotional origin of peptic ulcer, despite all that has been written on the subject, remains *sub judice*. Kellock recently could find no evidence of an "ulcer personality" in 250 persons with duodenal ulcer when he studied their childhood histories in great detail.

Certainly, for the clinician, the obvious task is to remain observant, and he will do well to remember that, even after the most searching examination reveals no lesion, it does not necessarily follow that a lesion does not exist. When a perversion of function can be proved, it does not necessarily follow that the functional alteration is due to emotional stress. Almost everyone is under stress and this fact tends to rob the current psychosomatic trend of much of its validity. Today there are dermatologists who believe that *almost* every lesion they see is dermatitis nervosa. Today there are dentists who have taken up the hue-and-cry of psychosomatacism as if it were an extremely smart and modern twist, and actually believe that pyorrhea is a result of psychic shock. Where will this tendency end?

The psychiatrists have a job to do and so also have the neurologists and internists. At present the two branches of investigation do not seem to be particularly miscible. It is silly to carry the psychosomatic concept too far and attribute everything upon and under the skin to emotional stress.

HAVE WE FORSAKEN GASTRIC ANALYSIS?

Two decades ago a great many clinics and clinicians routinely employed simple or fractional gastric analysis particularly on patients who 1) showed *any* kind of anemia or who 2) complained of *any* kind of indigestion. Today, at least the majority of internists reserve the procedure as a possible means of distinguishing between gastric ulcer and gastric carcinoma, although we know that some cases of cancer of the stomach present normal levels of gastric acidity and that some cases of ulcer of the stomach present low acidities at the time of examination.

Possibly there is such an entity as hyperacidity without ulcer and without gastritis and in such cases gastric analysis furnishes the clue to treatment, viz., alkalinization. On the other hand, while we are aware that a large percentage of adults have achlorhydria (after the Ewald or alcohol meal), how much do we know about the possible symptoms attributable to achlorhydria, *per se?*

Many physicians have written on this subject and it seems probable that not a few persons with indigestion, who show such achlorhydria, are greatly improved by taking dilute hydrochloric acid with and/or after meals. Clark (1) recently reported 67 cases in which the following symptoms were present,—gas, belching, epigastric pain and pressure, diarrhea, nausea, burning, secondary anemia, as well as "sour" stomach, excessive flatus, pain in the right lower quadrant, and constipation. All had achlorhydria in response to the alcohol meal, and in all cases, the symptoms disappeared after the use of dilute hydrochloric acid. Further studies such as Clark's are indicated. It is possible, and even probable, that we have been too careless in so greatly restricting the use of gastric analysis as a diagnostic procedure.

1. Clark, G. E.: Symptomatology of achlorhydria. Texas State J. Med., 48, 3, March 1952, 125-126.

ANTIBIOTICS AND ACTH IN CANCER

Maguire and McElhone (1) of Sydney, Australia, have produced remarkable changes in patients with cancer by using a combination of aureomycin and ACTH. Even moribund patients have, according to their report, shown very rapid general improvement, some of them even returning to their usual occupations while taking maintenance doses of both drugs. The malignant lesions have regressed but, thus far, none have completely disappeared. This striking work deserves consideration and duplication. Rather large doses of the antibiotic are used in the beginning of treatment, and a considerable dose of ACTH is continued throughout. The work is based on the theory that the course of cancer is a virus which gains access to the interior of the cell, and that it can be rendered accessible to the antibiotic through the use of ACTH. We will await with interest for further reports from these investigations.

1. Med. J. Australia, Dec. 8, 1951, 769-772.

BOOK REVIEWS

PHYSIOLOGICAL CHANGES IN PLASMA PROTEINS CHARACTERISTIC OF HUMAN REPRODUCTION. Icie G. Macy, Ph. D., Sc. D. and Harold C. Mack, M. D. Published by the Children's Fund of Michigan, 600 Frederick St., Detroit 2, Michigan. (Gratis).

This book of 170 pages deals principally with changes in the blood proteins which occur in women during pregnancy. The alterations in the plasma albumin and the various globulins have been estimated by means of electrophoresis and, in many cases, the results were checked against precipitation methods. The results obtained by the authors unquestionably furnish very valuable basic and reference material for all workers interested in this subject. Ralph E. Sloan, Editor of the Research Laboratory, states that copies may be obtained merely by writing to him at the address given above.

RECENT ADVANCES IN CLINICAL PATHOLOGY. Edited by S. C. Dyke, D. M. (Oxon) F.R.C.P. (London). The Blakiston Company, Philadelphia, $6.00.

This is the second edition of a popular English text and has been almost completely rewritten to include the most important advances in clinical pathology. It is written by 32 very well-known clinical pathologists and is divided into five sections,—Bacteriology, Biochemistry, Hematology, Histology, and Laboratory design and equipment. New material is included on infections, skeletal diseases, diabetic coma, paper chromatography, hemolytic and deficiency anemias and biopsy material. The book is well illustrated. The colored plates in the chapter on The Deficiency Anemias are excellent.

PROGRESS IN ALLERGY. Edited by Paul Kallos. Interscience Publishers, Inc., 250 Fifth Ave., New York, N. Y. $16.50.

This ponderous volume, running to nearly 600 pages, touches the problems of allergy in a special way. The introduction by Kallos, himself, is interesting and up-to-the-minute. Subjects dealt with by contributing authors include—allergy in children, bronchial asthma, bronchial asthma due to food allergy (by Rowe), the infective factor in asthma and rhinitis, cardiovascular allergy, a clinical investigation of the ergot alkaloids, drug allergy and, finally, sedormid purpura,—an immunological study of a form of drug hypersensitivity. The book should prove valuable not only to the allergist but the general practitioner and internist.

THE MERCK INDEX OF CHEMICALS AND DRUGS. Merck & Co., Inc., Rahway, N. J., 1952. $7.50.

This is the Sixth Edition of the Index,—an encyclopedia for the chemist, pharmacist, physician and allied professions. The 1167 pages of text deal chiefly with descriptions of substances in alphabetical order. Each entry indicates the preferred chemical name or other chemical names, common or popular names, generic and trade names. Trade-mark listing is as complete as possible and cross-indexed. Properties and sources of substances, preceded by methods of preparation, with literature references are given, with an indication of uses. Hazards and toxicities are mentioned. Indication is given of commercial availability and grades, such as U.S.P., N.F. and reagent. Empirical formulas, molecular weights and percentage composition are given for all chemicals having a definite structure. No one with an interest in pharmacology, chemistry or physiology can afford to be without this Index. The thumb-index edition is $8.00.

GENERAL ABSTRACTS OF CURRENT LITERATURE

BADENOCH, J.: *Cortisone in idiopathic steatorrhea.* Brit. Med. J., Feb. 16, 1952, 356-357.

Four patients with idiopathic steatorrhea, two of them previously untreated, were given 100 mg. of cortisone daily, intramuscularly for four weeks. Although the fat excretion decreased in all, the difference was not statistically significant, and in none was the steatorrhea abolished. It would therefore appear that adrenal cortical function is normal in cases of steatorrhea.

SWYNNERTON, B. F. AND TRUELOVE, S. C.: *Carcinoma of the stomach.* Brit. Med. J., Feb. 9, 1952, 287-292.

From 1938-49, 375 patients with gastric cancer were treated at the Radcliffe Infirmary, Oxford. There were almost twice as many men as women, and in each sex the peak incidence was in the sixties. The commonest site of the lesion was pre-pyloric. In 25 percent of patients, gastric acidity was normal or high. Resection offers the only hope of prolonged life. Among the resected group the chief factor influencing prognosis was the length of illness prior to operation. The prognosis was unexpectedly found to improve with increasing length of history, a fact due to the occurrence of slow growing cancers.

MORGAN, R. H. AND STEWART, H. B.: *Clinical experience with Telepaque, a new gallbladder compound.* Radiology, 58, 2, Feb. 1952, 231-234.

In a quantitative investigation of the characteristics of a new gallbladder compound, Telepaque, (Winthrop-

Stearns, Inc.) the clarity of the gallbladder visualization was found to be about 35 percent greater than that obtained with iodoalphionic acid. About 40 percent fewer individuals required a double dose of Telepaque for satisfactory visualization than was the case with iodoalphionic acid. Very few side reactions occurred. Among 100 patients, none experienced vomiting, only a few complained of nausea (mostly of a very slight nature) and the great majority had no symptoms of any kind.

WEBSTER, D. D. AND BLADES, A. N.: *Islet-cell tumor of the pancreas.* Brit. Med. J., Feb. 9, 1952, 307-308.

A fatal case of functioning insuloma of the pancreas in a woman of 20 is described. An unusual feature was the regular association of symptoms with the beginning of the menstrual period. After 3 or 4 monthly attacks, which might easily have been regarded as psychomotor epilepsy, she went into a deep coma with a blood sugar of 64 mg. percent, and failed to respond to intravenous glucose. The pituitary gland was enlarged 50 percent. The pancreas contained a rounded dark red, soft-solid tumor ½ inch in diameter projecting forward from the gland at the junction of the middle and left thirds. It consisted of solid islet tissue, had no capsule, and was invading blood vessels, but since no metastases were found, it is impossible to say that it was malignant. The authors suggest that in a case of this kind, in coma, and unresponsive to glucose, that a small dose of insulin (10-15 units) be given following the glucose, as it apparently acts as a cerebral stimulant in comparable situations sometimes encountered during insulin-shock therapy.

CATHIE, I. A. B. AND MACFARLAND, S. C. W.: *Incidence of Bact. Coli O group III in sporadic infantile gastro-enteritis.* Brit. Med. J., Oct. 27, 1951, 1002-1004.

Figures are presented of the isolation of *Bact. Coli* O group III (D433) from 264 cases of sporadic infantile gastro-enteritis over a 2 year period. In a shorter control series, this organism was isolated as often as in cases of the disease. No correlation was found between the total number of cases notified to the London County Council Health Dept. and the percentage of cases in which the organism was found on admission to the Hospital for Sick Children.

BROWN, R. L.: *The gastrointestinal tract and stool following aureomycin therapy.* Antibiotics and Chemotherapy, 2, 1, Jan. 1952, 5-11.

Aureomycin in therapeutic doses (1 to 1¼ Gm. daily) not infrequently causes mild but significant gastrointestinal symptoms and signs; these include chemical, fungal and hypovitaminosis B glossitis, stomatitis, pharyngitis, esophagitis, gastritis, colitis and proctitis. These symptoms and signs disappear within days after discontinuance of therapy. Hypovitaminosis B (and Candidiasis lesions) respond to specific therapy or improve with time. The uncomplicated aureomycin lesion is characterized by hyperemia, mild edema, and brown discolorations. The hypovitaminosis lesion is characterized by early development of erythema with frequently associated fissures, the candidiasis lesions were often recurrent and classical. Symptoms were nausea, glossodynia, oropharyngodynia, epigastric distress, colon tenderness and rectalgia. Several bulky, loose stools occurred during treatment, but became compact and scybalous within several days. Microscopically, the feces showed no abnormal nor increased cell count. In positive cases, Candida was increased.

ROCCO, A. F., HUNT, R. R. AND SAVRAN, J.: *Acute pneumocholecystitis.* Radiology, 58, 2, Feb. 1952, 228-230.

A case of pneumocholecystitis in a 45-year-old colored female is presented. The diagnosis can be made only by x-ray films and shows gas or air in the gall bladder and gas also in the gall bladder wall. Only 18 cases had been previously reported. Occasionally *Clostridium Welchii* or *perfringens* infection is responsible for the gas. Usually, no explanation is available. Scout films should be made of the gall bladder where the condition is suspected. After the gas had largely absorbed, the gall bladder was removed with some difficulty because of adhesions and it showed an edematous mucosa and contained 100 tiny morulated stones.

MCDONOUGH, F. E., HEFFERNON, E. W. AND PATTERSON, M.: *Chronic relapsing pancreatitis.* Am. Pract. & Dig. Treat., 2, 11, Nov. 1951, 928-932.

Chronic relapsing pancreatitis has become firmly established as a disease entity with characteristic clinical manifestations and pathological findings. Diagnosis remains difficult because the severity and frequency of the attacks are unpredictable and cumulative pancreatic damage is variable. Severe abdominal pain contrasted with minimal physical signs is the most helpful diagnostic criterion. Both medical and surgical treatment is unsatisfactory. The absence of physical signs between attacks also has diagnostic value. Unless calcification of the pancreas, diabetes, cyst formation, extreme enlargement of the head of the pancreas or steatorrhea have developed, there is no way to suspect the disease, between attacks, except for the history of recurring abdominal pain.

BENSON, R. A., SCHWARTZMAN, J., GREEN, M. AND REED, H. W.: *Banthine in pediatrics: a preliminary report on sensitivity, toxicity and dosage.* Arch. Ped. 68, 9, Sept. 1951, 420-426.

Skin tests and conjunctival tests were of no value in detecting untoward reactions to Banthine. Children tolerate the drug well in suitable doses and no blood, renal or hepatic disturbances were observed. Parenteral medication should not exceed half the recommended oral dose since it resulted in more severe reactions. Banthine did not show much promise in pyloric stenosis but may prove to be of value as a supplementary measure in vomiting and diarrhea. A wider trial is necessary. Most infants tolerated a dosage of 12.5 mg. from once to three times daily by mouth.

ROSE, T. F.: *Pancreatitis at the Royal North Shore Hospital of Sydney from 1925 to 1950.* Med. J. Australia, Oct. 6, 1951, 453-458.

Rose takes the logical view that pancreatitis is pancreatitis, whether it is acute, interstitial, acute hemorr-

hagic, acute or subacute recurring, or chronic recurrent over a period of years. Any form may produce abscess or pseudocyst. Over a 25 year period among 156,500 admissions, there were 89 cases of pancreatitis.

In 69 cases the attacks were acute. Only 20 cases were of the chronic recurrent type in whom, incidentally, pancreatic lithiasis was not observed. Two autopsies on patients with chronic recurrent pancreatitis showed pancreatic fibrosis. Four who lived from 3 to 10 years all died in an attack of acute pancreatic edema (interstitial) with fat necrosis. This acute phase seen in current cases links them with the acute cases. Disturbances in carbohydrate metabolism vary from transient glycosuria to full-blown diabetes. Association with cholecystitis or hepatitis is not uncommon. Cholecystectomy is undesirable in these cases as it carries an extremely high mortality (80 percent in this series). The present vogue in treatment is conservative, with no surgical interference. However, the results of treatment depend more on the severity of the disease than the form of treatment.

CARROLL, C., FETICK, P. G. AND LYNXWILER, C. P.: *Bacillary dysentery: report of case.* Arch. Ped., 68, 12, Dec. 1951, 589-592.

The authors report a typical case of bacillary dysentery occurring in a four-year-old child. Fever, toxicity, tenesmus, abdominal pain, and frequent stools containing mucus, blood and pus are the chief features. Shigella dysenteriae of Flexner was isolated from the stools. Excellent results were obtained by the use of chloromycetin. This drug is much less toxic than the sulfonamides.

JOHNSTON, M. W., SQUIRES, A. H. AND FARQUHARSON, R. F.: *The effect of prolonged administration of thyroid.* Ann. Int. Med., 35, 5, Nov. 1951, 1008-1022.

The internal secretion of the thyroid gland may be greatly decreased and the gland reduced in activity and put at rest by the presence in the body of excessive amounts of its specific hormone. Most euthyroid patients are able to acquire tolerance to the ingestion of large amounts of thyroid. Great individual variation is found in the degree of tolerance attained. One patient took 10 gr. daily with impunity, while another was unable to tolerate 3 grains. The tolerance developed on a large dose is associated with a gradual decrease in the serum protein-precipitable iodine and in the basal metabolic rate. The ingestion of excessive amounts of thyroid hormone over a long period does not appear to affect the gland permanently. The depression is reversible and, once thyroid therapy is stopped, normal activity is soon regained.

LAURENS, J.: *Ano-rectal problems in the home and office.* J. Arkansas Med. Soc., XLVIII, 7, Dec. 1951, 151-154.

Laurens seldom recommends the performance of rectal surgery on the ambulatory patient, yet there are many conditions which may be treated in the home or office. The acute external thrombotic hemorrhoid, if seen early, should have the clot extracted. Thrombectomy is occasionally required. In protruding, massive, edematous, thrombosed hemorrhoids, hospitalization is advisable for definitive surgery. In anal fissure the injection of the base with an oil-soluble anesthetic usually produces enough relaxation of the sphincter to permit healing. Cryptitis is the basis for almost all benign ano-rectal disease. Crypts may, when necessary, be opened in the office, using a crypt hook whose concave margin is sharpened. If papillitis is present, hospitalization is advised. The problems of fecal impaction and rectal bleeding also are considered.

HALL, A. A. AND MASEN, J. M.: *A test for the more accurate recognition of gall bladder and liver bile during diagnostic biliary drainage.* Ann. Int. Med., 35, 4, 812-819.

In order to facilitate making the distinction between gall bladder bile and liver bile, the authors employed the iodine compound priodax. Priodax was present in the liver at the end of 14 hours but not at the end of 38 hours. Although the experienced observer can distinguish among A, B, and C Biles, coming respectively from the common duct, gall bladder and liver, the iodine test, which the authors have investigated, makes the distinction much more accurate.

WHITFIELD, A. G. W AND ARNOTT, W. M.: *Transient disturbances of consciousness in hepatic cirrhosis.* Brit. Med. J., Nov. 3, 1951, 1054-1056.

Transient attacks of unconsciousness are described in three cases of hepatic cirrhosis. Death in cholemia followed within a period of months in two of the cases. The attacks were associated with fluid retention but the precise mechanism of their causation is not clear. The attacks lasted from hours to days and were sometimes temporarily regarded as strokes. Cardiological and neurological findings were nil, although abnormal ECG's were obtained.

KELLOCK, T. D.: *Childhood factors in duodenal ulcer.* Brit. Med. J., Nov. 10, 1951, 1117-1120.

Kellock investigated the childhood histories of 250 men with duodenal ulcer and compared them with an equal number of controls suffering from various other diseases. Family and parental factors, education, childhood illnesses and social class were very carefully detailed, but no difference could be found between the 2 groups in any of the points investigated. He admits that factors operating in late adolescence or early adult life, had these been investigated, might be responsible for the so-called "ulcer personality." He thinks possibly the ulcer personality may be the product of the disease, rather than the reverse.

FINNEY, G. E.: *Duodenal ulcers: place of surgical treatment.* Texas State Med. J., 47, 11, Nov. 1951, 756-759.

Relying on personal experience, Finney emphasizes the need for individualizing the treatment in every case to suit the patient's needs. When possible, without too much risk, subtotal gastrectomy is probably the operation of choice. However, vagus resection with a posterior gastroenterostomy or adequate pyloroplasty have proved satisfactory. He has had very little trouble due to vagotomy except transient diarrhea.

AMER. JOUR. DIG. DIS.

THE AMERICAN CONGRESS OF PHYSICAL MEDICINE

The 30th annual scientific and clinical session of the American Congress of Physical Medicine will be held on August 25, 26, 27, 28 and 29, 1952 inclusive, at The Roosevelt Hotel, New York, N. Y. Scientific and clinical sessions will be given on the days of August 25, 26, 27, 28 and 29. All sessions will be open to members of the medical profession in good standing with the American Medical Association. In addition to the scientific sessions, annual instruction seminars will be held. These lectures will be open to physicians as well as to therapists, who are registered with the American Registry of Physical Therapists or the American Occupational Therapy Association. Full information may be obtained by writing to the American Congress of Physical Medicine, 30 North Michigan Avenue, Chicago 2, Illinois.

DRUG-MAKERS CITED FOR CONSISTENT PRICE REDUCTIONS

Medical and pharmaceutical research, which has made its greatest strides in the conquest of bacterial infections, will be chiefly directed in the coming years to "unlocking the mysteries of endocrinology, cancer and neurological and mental disturbances," according to Dr. Theodore G. Klumpp, president of Winthrop-Stearns Inc., pharmaceutical manufacturer, and director, World Medical Association.

Dr. Klumpp hailed the "hand-in-hand" progress of medical and pharmaceutical research in an address May 14th at the Rutgers Pharmaceutical Conference, held on the campus of Rutgers University. Theme of the conference, which was sponsored by the College of Pharmacy and University Extension Division, was "Progress Through Knowledge and Mutual Understanding." It was attended by educators, drug manufacturers, wholesalers and retailers, and members of allied health professions.

Noting that medicine and pharmacy are now able to treat the bulk of known bacterial infections, Dr. Klumpp forecast that the virtually untouched field of virus diseases will "next yield to the magic wand of research.

"But I believe that the greatest activity in our industry will turn toward three other fields of interest: endocrinology, cancer, and neurological and mental disturbances.

"In endocrinology we have a host of glandular disturbances and imbalances, and such unsolved disease entities as diabetes. I believe that high blood pressure and arteriosclerosis, the latter the greatest killer of them all, belong in this category.

"We have no real clue as to the nature of cancer and it is possible that several distinct and different mechanisms will prove to be the cause. It is even more likely that many serious and unsolved mental and neurological disturbances will prove to be different in their origins and causative mechanisms," Dr. Klumpp said.

Dr. Klumpp cited the pharmaceutical industry's "phenomenal growth" during the past 35 years in providing the "tools of medicine."

"We have converted the penicillin mold from a laboratory curiosity to the greatest life-saving drug the world has thus far known . . . and I might also mention that our industry brought the price down from $40 for 100,000 units in 1942 to one and eight-tenths cents for the same amount in 1952.

"This is eloquent evidence of the competence of our research and of the vitality of competition in our industry."

In 1947, Dr. Klumpp pointed out, the sales of pharmaceutical preparations to U. S. physicians alone amounted to $577 million. This figure is estimated to have risen to $1 billion in 1951.

"It is significant that this gain is real and not the result of inflation, since pharmaceutical prices have not risen since the end of World War II; in fact, they have declined considerably. No industry has done a better job in this respect, to my knowledge."

Commenting on the complaints of some consumers that medicine prices are "too high," Dr. Klumpp said that "there is a great need in the medical profession and drug industry to make the public appreciate that good medical care is not only worth the price, but worth a lot more than candy or cigarettes or a television set."

SOCIALIZED MEDICINE IN BRITAIN

Britain's four year experiment in socialized medicine has been described in the June 16th issue of The Freeman as a "Utopia in a Straitjacket" which has stifled medical research, degraded the doctor and virtually bankrupted the nation.

Melchior Palyi, who conducted a survey of Britain's national health program for the publication, says:

"This something-for-nothing Utopia, advertised world-wide, is now in slow retreat; the Labor government itself set a ceiling of $1,100,000,000 on direct medical expenditures; and the conservative government is attempting to enforce it by making the patient pay roughly one-half the cost of dentures and eyeglasses, and a small fee for each prescription."

Palyi, author of "Medical Care and the Welfare State," says that in less than three years doctors wrote 609 million prescriptions, fitted 19,500,000 pairs of glasses and 130,000 hearing aids and dentists worked on seven million dentures. He added:

"The overall state of general practice is bad and still deteriorating and has reached the point where, despite the efforts of the most conscientious individual doctors, it is at best a very unsatisfactory medical service and at the worst a positive source of public danger.

"The nation's health is actually jeopardized because the exorbitant cost of over-extended curative medicine forces the shelving of urgently needed outlays to prevent the occurrence and spread of disease."

The author also asserted that under the National Health Program his on-the-spot survey showed that families are abandoning the old and the defective, who, he added, are filling the hospitals. Palyi continued:

"The public is adopting the attitude that because of the Welfare State they have no responsibilities for their aged parents."

The Freeman quoted Palyi as saying that the costs of governmentalized medicine have trebled in four years to more than 19 per cent of the national budget and added:

"Yet new hospitals are conspicuously non-existent, and the enlargement of old ones is negligible, though the lack of adequate facilities was one of the major arguments for the new scheme."

The Freeman article pointed out that under the national health plan, doctors in industrial centers are

the purging parent...

She boasts of keeping the poor child's bowels "wide open."

Instead of keeping the youngster healthy, she is establishing a laxative limp in the digestive tract.

Turicum, giving ***lubricoid action without oil,*** affords sane therapy without irritating the bowel.

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each burdened with 3,000 to 4,000 and even more registered patients.

It said that this means that a doctor has three minutes or less for a consultation with his patient during which time he should diagnose and advise, make out an ever-increasing number of prescriptions, write letters to specialists and hospitals and fill out many forms of official documents.

Palyi said that within the first three years of operation more than every second Britisher has received free dental treatment. And he warned:

"But the dentists, rushed from one chair to another, are forced to sacrifice the dental care of school children whose teeth are decaying."

The Freeman stressed that under the national health program many deserving cases are obliged to wait as much as 12 months for a hospital bed because of crowded conditions and added:

"The core of the problem is the perversion of the doctor-patient relationship into a silent conspiracy to provide both partners with mutual advantages at the expense of the scheme.

"It is generally understood that deals between drug producers and panel doctors are a widespread practice."

(Reprints of the survey in booklet form are available from The Freeman, 240 Madison Avenue, New York 16, N. Y. at ten cents a copy.

SCORES OF GOOD JOBS IN PHARMACEUTICAL PROFESSION OPEN

There are scores of good jobs open for trained young men and women in the pharmaceutical profession, an executive of Parke, Davis & Company declared here today in a speech before the Florida State Pharmaceutical Association.

John A. MacCartney, trade relations manager of the world's largest pharmaceutical firm, said at the Sea Isle Hotel that the rate of graduates from schools of pharmacy continues at "a fairly-high level."

He emphasized, however, "Due to the interim of two world wars, pharmacists are dropping off the top of the chronologic ladder faster than they are climbing the bottom rung."

MacCartney told the Florida

pharmacists they "should get a volume of business today which exceeds your wildest expectations of even 10 years ago."

He pointed out, "All this requires more trained help."

MacCartney urged the pharmacists to keep uppermost "the problem of getting professional employes who are capable of handling the highly technical services which you, as responsible professional pharmacists, must render to your community, if you expect to grow in business."

EYE LOTION WITH NEO-SYNEPHRINE INTRODUCED BY WINTHROP-STEARNS

OpH, a new eye lotion with Neo-Synephrine hydrochloride, providing safe, dependable decongestive action, has been introduced by Winthrop-Stearns Inc., it was announced by Dr. Theodore G. Klumpp, president.

A unique feature of OpH is the container which is so constructed that it also serves as the dropper; a separate eye-dropper is not needed. This rules out the possibility of contaminating the lotion, as can readily happen with the conventional type of eye-dropper which is constantly inserted in the lotion.

OpH is decongestant, astringent and antiseptic. Affording soothing relief of minor eye irritations, including those from wind, sun and dust, it contains 0.08 per cent Neo-Synephrine hydrochloride, 0.06 per cent zinc sulfate and 2.2 per cent boric acid.

At this concentration, Neo-Synephrine produces prompt and prolonged decongestion with almost no mydriatic effect. No congestive rebound follows its use, nor do repeated applications weaken its effectiveness. Zinc sulfate, mildly astringent and antiseptic in action, is of value in reducing conjunctival irritations due to exposure. Boric acid, is well tolerated by the conjunctiva and cornea.

OpH is available in convenient applicator bottles of 10 cc. from Winthrop-Stearns Inc.

NEW FORM OF THEOMINAL HAS LESS SEDATIVE EFFECT

As a companion product to Theominal, the vasodilator antispasmodic drug, a new preparation called Theominal M, designed to produce less sedation, has now been made available, it was announced by Dr. Theodore G. Klumpp, president of Winthrop-Stearns Inc. Containing less than half as much Luminal as the original product, Theominal M is especially suitable in the treatment of hypertension cases where minimal sedation is desired.

Theominal and Theominal M are indicated for the control of high blood pressure in arteriosclerosis, angina pectoris, arterial hypertension and climacteric disorders. Both drugs are combinations of theobromine and Luminal in tablet form. They contain the same quantity, 0.32 Gm., of theobromine.

Theominal M, however, contains only 15 mg. of Luminal, as compared with 32 mg. in Theominal. The differing amounts of Luminal in the two products make it possible, for a given daily dose of theobromine, to achieve a desired balance between optimum sedation and minimum drowsiness.

selective control

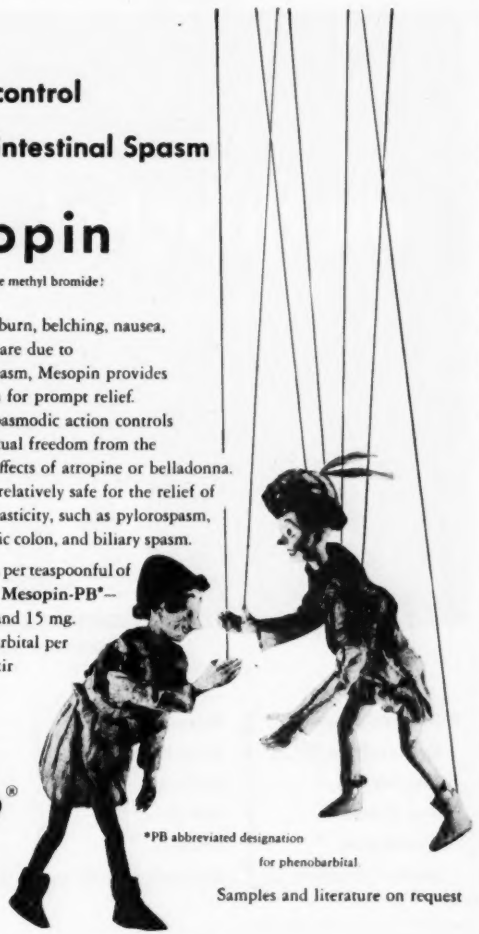
of Gastrointestinal Spasm

Mesopin

(brand of homatropine methyl bromide)

When pain, heartburn, belching, nausea, or unstable colon are due to gastrointestinal spasm, Mesopin provides an effective means for prompt relief. Its selective antispasmodic action controls spasticity with virtual freedom from the undesirable side effects of atropine or belladonna. Thus, Mesopin is relatively safe for the relief of gastrointestinal spasticity, such as pylorospasm, cardiospasm, spastic colon, and biliary spasm.

Mesopin—2.5 mg. per teaspoonful of elixir or per tablet. **Mesopin-PB***—2.5 mg. Mesopin and 15 mg. (1/4 gr.) phenobarbital per teaspoonful of elixir or per tablet.



Endo®

*PB abbreviated designation for phenobarbital.

Samples and literature on request

Endo Products, Inc., Richmond Hill 18, N. Y.

The effect of theobromine is to produce peripheral arterial dilatation, while Luminal relaxes the existing vascular spasm and allays nervous excitement. In combination, they tend to reduce blood pressure gradually and to keep it within moderate limits.

Both Theominal and Theominal M are available from Winthrop-Stearns Inc. in bottles of 100 and 500 tablets.

LOZENGES-ROCHE

Nutley, N. J.—Synkayvite-CB Lozenges 'Roche' are new, cherry-flavored, candy-like lozenges which provide Synkayvite (vitamin-K compound) for prevention of delayed hemorrhage, vitamin C for promotion of wound healing and B-complex factors for reinforcing the nutritional status of the patient. Synkayvite-CB Lozenges are especially valuable in young patients following tonsillectomy when swallowing may be difficult. Recent clinical studies, such as that of King (*Southern Medicine and Surgery, 113*:186, 1951) have stressed the value of vitamins K, C and B-complex in preoperative and postoperative care. Synkayvite-CB Lozenges provide these factors in highly convenient and pleasant dosage form. Chemically, Synkayvite (water-soluble vitamin K compound) is the tetrasodium salt of the diphosphoric acid ester of 2-methyl-1, 4-naphthohydroquinone.

AMINODROX

The S. E. Massengill Company announces its new Specialty Product, Tablets Aminodrox.

Aminodrox is a combination of aminophylline and aluminum hydroxide gel, dried, prepared in five combinations. All combinations are uncoated tablets. This product provides aminophylline which is well tolerated by the oral route, and therapeutically effective blood levels can be established and maintained over prolonged periods of time without gastric upset. The usefulness of aminophylline is increased considerably when administered in Aminodrox, and offers the physician a convenient and dependable product.

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INOSITOL	0.083 Gm.	0.75 Gm.
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LIVER CONCENTRATE AND DESICCATED LIVER**	0.087 Gm.	0.78 Gm.

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**in 3 tablespoonfuls Syrup as 1.2 Gm. liver concentrate

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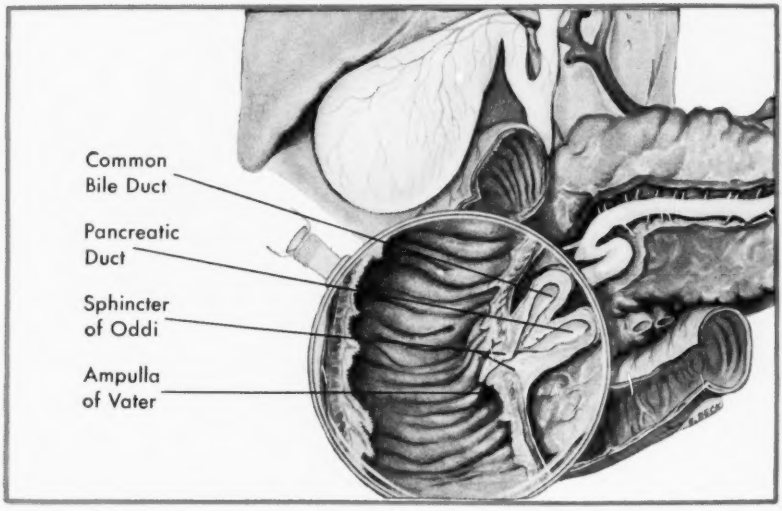

Bile Acids Therapy in Biliary Stasis . . . Biliary Dyskinesia

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KETOCHOL contains all four unconjugated bile acids—not salts. Its action on the hepatic cells results in an increase of thin, free-flowing bile which "flushes" and drains the hepatic tree.

Average dosage—one tablet three times a day, with meals.

PAVATRINE® with phenobarbital

—efficient antispasmodic used in conjunction with Ketochol in the medical management of gallbladder dysfunction.

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